Rofecoxib is a potent inhibitor of cytochrome P450 1A2: studies with tizanidine and caffeine in healthy subjects

Janne T. Backman, Marjo J. Karjalainen, Mikko Neuvonen, Jouko Laitila & Pertti J. Neuvonen

Department of Clinical Pharmacology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland

Correspondence

Dr Janne Backman MD, Department of Clinical Pharmacology, University of Helsinki, Haartmaninkatu 4, FIN-00290, Helsinki, Finland.

Tel: + 358 9 4717 3914

Fax: + 358 9 4717 4039

E-mail: janne.backman@hus.fi

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Aims

Case reports suggest an interaction between rofecoxib and the CYP1A2 substrate tizanidine. Our objectives were to explore the extent and mechanism of this possible interaction and to determine the CYP1A2 inhibitory potency of rofecoxib.

Methods

In a randomized, double-blind, two-phase cross-over study, nine healthy subjects took 25 mg rofecoxib or placebo daily for 4 days and, on day 4, each ingested 4 mg tizanidine. Plasma concentrations and the urinary excretion of tizanidine, its metabolites (M) and rofecoxib, and pharmacodynamic variables were measured up to 24 h. On day 3, a caffeine test was performed to estimate CYP1A2 activity.

Results

Rofecoxib increased the area under the plasma concentration–time curve ($AUC_{0-\infty}$) of tizanidine by 13.6-fold [95% confidence interval (CI) 8.0, 15.6; $P < 0.001$], peak plasma concentration (C_{max}) by 6.1-fold (4.8, 7.3; $P < 0.001$) and elimination half-life ($t_{1/2}$) from 1.6 to 3.0 h ($P < 0.001$). Consequently, rofecoxib markedly increased the blood pressure-lowering and sedative effects of tizanidine ($P < 0.05$). Rofecoxib increased several fold the tizanidine/M-3 and tizanidine/M-4 ratios in plasma and urine and the tizanidine/M-5, tizanidine/M-9 and tizanidine/M-10 ratios in urine ($P < 0.05$). In addition, it increased the plasma caffeine/paraxanthine ratio by 2.4-fold (95% CI 1.4, 3.4; $P = 0.008$) and this ratio correlated with the tizanidine/metabolite ratios. Finally, the AUC_{0-25} of rofecoxib correlated with the placebo phase caffeine/paraxanthine ratio ($r = 0.80$, $P = 0.01$).

Conclusions

Rofecoxib is a potent inhibitor of CYP1A2 and it greatly increases the plasma concentrations and adverse effects of tizanidine. The findings suggest that rofecoxib itself is also metabolized by CYP1A2, raising concerns about interactions between rofecoxib and other CYP1A2 substrate and inhibitor drugs.

Introduction

Recent case reports have suggested an interaction between rofecoxib and tizanidine [1, 2]. The adverse reactions observed involved both the central nervous and cardiovascular systems. However, the likelihood and mechanism of this possible interaction have not been documented in controlled studies.

Rofecoxib is a COX-2 selective nonsteroidal anti-inflammatory drug (NSAID). Prior to its possibly temporary withdrawal, due to an increased risk of myocardial infarction and stroke [3], rofecoxib was one of the most widely used coxibs. Its daily dose ranged from 12.5 to 50 mg and the drug could be taken once daily due to its long elimination half-life of about 20 h. Rofe-

Figure 1
Pathways of tizanidine metabolism [9]

coxib is extensively metabolized in the liver, probably by enzymes other than cytochromes P450 [4, 5]. According to a recent study, rofecoxib may be a modest inhibitor of CYP1A2 in humans [6].

Tizanidine is a centrally acting muscle relaxant, which has sedative and blood pressure-lowering properties. It undergoes extensive first-pass metabolism, its oral bioavailability being about 10–30% [7, 8], and it is metabolized down several pathways (Figure 1) [9]. CYP1A2 catalyses the elimination of tizanidine both *in vitro* and *in vivo* [10, 11]. Tizanidine can be more sensitive than theophylline and caffeine as a probe substrate for CYP1A2 in detecting small or transient effects on its activity, particularly during first-pass metabolism [8, 11].

Our aims were to study the effect of rofecoxib on CYP1A2 activity in humans, using tizanidine and caffeine as probe substrates, and to investigate the mechanism and consequences of a possible rofecoxib–tizanidine interaction.

Methods

Subjects

Nine healthy male nonsmokers (age range 20–25 years; weight range 64–87 kg) completed the study (Table 1). One subject withdrew from the study for personal reasons before the first tizanidine dose. Male subjects were chosen to avoid possible effects of the menstrual cycle on tizanidine pharmacokinetics. Prior to enrolment, subjects were ascertained to be healthy by a physical examination, a 12-lead electrocardiogram and routine

laboratory tests. Subjects with a systolic blood pressure <110 mmHg were excluded for safety reasons. All subjects provided written informed consent before the physical examination.

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, Helsinki, Finland. It was carried out according to a randomized, double-blind, two-phase cross-over design with a 2-week wash-out period. The subjects took 25 mg rofecoxib (one 25-mg Vioxx tablet; Merck & Co, Whitehouse Station, NJ, USA) or placebo once daily at 08.00 h for 4 days. On day 4, they ingested a single oral dose of 4 mg tizanidine (one 4-mg Sirdalud tablet; Novartis Pharma, Wehr, Germany) with 150 ml water at 09.00 h. A standard meal was served 3 and 7 h after tizanidine ingestion. Subjects were not allowed to drink grapefruit juice for 1 week before each study day. Alcohol and drinks containing caffeine were not permitted on the study days.

The subjects were under close medical supervision during the days of tizanidine administration. Fluids for intravenous infusion were available for immediate use, but were not needed.

Sampling

On the days of administration of tizanidine, a forearm vein of each subject was cannulated with a plastic can-

Table 1

Characteristics of the subjects, the pharmacokinetic variables for rofecoxib, and the results of the caffeine test

Subject number	Age, years	Weight, kg	C_{max} , ng ml ⁻¹	Rofecoxib		$t_{1/2}$, h	Caffeine/paraxanthine ratio	
				AUC_{0-25h} , ng h ml ⁻¹	$AUC_{0-\infty}$, ng h ml ⁻¹		During placebo (control)	During rofecoxib
2	21	84	367	5453	8846	9.1	1.66	3.74
3	22	80	243	3271	4332	10.9	0.89	1.58
4	21	83	196	2905	4347	14.4	0.91	2.37
5	22	80	320	4043	6700	18.2	1.26	3.58
6	21	73	302	3906	5374	12.3	1.45	2.78
7	23	71	324	4396	7929	20.9	1.75	7.66
8	25	87	331	5885	18818	40.0	3.26	7.79
9	20	64	407	4891	9648	24.6	1.32	1.81
10	22	86	241	3949	12490	44.2	1.66	2.38
Mean	22	79	303	4300	8721	21.6	1.57	3.74*
SD	1	6	66	971	4628	12.7	0.70	2.37

AUC_{0-25h} , Area under plasma concentration–time curve from time 0–25 h; $AUC_{0-\infty}$, area under plasma concentration–time curve from time 0 to infinity; C_{max} , maximum plasma concentration; $t_{1/2}$, half-life. * $P = 0.008$, vs. control.

nula 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 drawn to ethylenediaminetetraacetic acid-containing tubes. Plasma was separated within 30 min and stored at -40°C until analysis. Urine was collected cumulatively in two fractions, 0–12 h and 12–24 h. The samples were stored at -40°C for later analysis.

Assessment of CYP1A2 activity

A caffeine test was performed on the third day of pre-treatment during both phases [12–14]. The subjects ingested 100 mg caffeine (one 100-mg Cofi-Tabs tablet; Vitabalans, Hämeenlinna, Finland) at 09.00 h, after having abstained from caffeine intake for at least 12 h. A blood sample for the analysis of plasma caffeine and paraxanthine (1,7-dimethylxanthine) concentrations was taken from each subject 6 h after caffeine intake.

Determination of drug concentrations in plasma and urine

Plasma and urine concentrations of unchanged tizanidine and its metabolites (M) were quantified using 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 Corp.; Milford, MA, USA) using gradient elution. The mobile phase consisted of 10 mmol l⁻¹ ammonium acetate (pH 9.5,

adjusted with 25% ammonia solution) and acetonitrile. The mass spectrometer was operated in the atmospheric pressure ionization 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⁻¹ and the day-to-day coefficient of variation (CV) was 5.6% at 0.096 ng ml⁻¹, 3.1% at 0.96 ng ml⁻¹ and 4.6% at 9.6 ng ml⁻¹ ($n = 7$). 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. The CVs were between 3 and 11% for the different tizanidine metabolites at relevant concentrations ($n = 8$). Rofecoxib did not interfere with the analysis of tizanidine and its metabolites.

The plasma concentrations of rofecoxib were determined by high-performance liquid chromatography (HPLC) with etoricoxib as the internal standard [15]. The limit of quantification was 5 ng ml⁻¹ and the day-to-day CVs were 0.9% at 20 ng ml⁻¹, 4.1% at 100 ng

ml⁻¹ and 0.6% at 400 ng ml⁻¹ ($n = 6$). Tizanidine did not interfere with the determination of rofecoxib in plasma.

Plasma caffeine and paraxanthine concentrations were determined by HPLC with ultraviolet detection, with [beta]-hydroxy-ethyltheophylline as the internal standard [16, 17]. The limit of quantification was 50 ng ml⁻¹ for both compounds. The within-day CV for caffeine and paraxanthine was <6% at relevant concentrations ($n = 7$).

The sources of the reference compounds were: tizanidine hydrochloride (Farmak, Olomouc, Czech Republic), rofecoxib (Merck & Co., and Sequoia Research Products Ltd, Pangbourne, UK), clonidine hydrochloride (Boehringer Ingelheim GmbH, Ingelheim, Germany), caffeine (Sigma-Aldrich Corp., St Louis, MO, USA), paraxanthine (Sigma-Aldrich Corp.) and etoricoxib (Merck & Co.).

Pharmacokinetics

The pharmacokinetics of tizanidine and its metabolites were evaluated by the peak concentration in plasma ($C_{\max}$), time to $C_{\max}$ ($t_{\max}$), area under the plasma concentration–time curve (AUC) from 0 to 24 h (AUC_{0-24}) and to infinity ($AUC_{0-\infty}$), and elimination half-life ($t_{1/2}$). The terminal log-linear part of the concentration–time curve was identified visually for each subject. The elimination rate constant (k_e) was determined with the use of linear regression analysis of the log-linear part of the plasma concentration–time curve. The $t_{1/2}$ was calculated by the following equation: $t_{1/2} = \ln 2/k_e$. The AUC values were calculated by use of the linear trapezoidal rule for the rising phase of the plasma drug concentration–time curve and the log-linear trapezoidal rule for the descending phase, with extrapolation to infinity, when appropriate, by division of the last measured concentration by k_e . The amount of tizanidine and its metabolites excreted into urine (A_e) was determined. The pharmacokinetics of rofecoxib were characterized by $C_{\max}$, $t_{\max}$, $t_{1/2}$, the AUC from 0 to 25 h after the last dose of rofecoxib (AUC_{0-25}) and the AUC from 0 to infinity ($AUC_{0-\infty}$). Of note, the extrapolated fraction of the $AUC_{0-\infty}$ of the tizanidine metabolite M-3 during the rofecoxib phase and that for rofecoxib exceeded 20%. The pharmacokinetic calculations were performed using the program MK-model, version 5.0 (Biosoft, Cambridge, UK).

Pharmacodynamics

The pharmacodynamic variables were assessed before administration of tizanidine and immediately after each blood sample was taken, up to 24 h after drug intake. The systolic and diastolic blood pressure and heart rate were measured twice from the forearm with the subject

in a sitting position and the mean value was used in the calculations. The blood pressures and heart rates were measured with an automatic oscillometric blood pressure monitor (HEM-711; Omron Healthcare GmbH, Hamburg, Germany). Before the beginning of the study, the subjects were trained to perform three psychomotor tests. In the Digit Symbol Substitution Test (DSST), the number of digits correctly substituted in 2 min was recorded [18]. Subjective drowsiness and drug effect were measured using a 100 mm long horizontal visual analogue scale (VAS) [19]. For each pharmacodynamic variable, the maximum response was recorded and the area under the effect vs. time curve from 0 to 12 h (AUC_{0-12}) was calculated using the trapezoidal rule. A 12-lead electrocardiogram (ECG) was recorded prior to and at 2 h after tizanidine intake.

Statistical analysis

All results are given as mean $\pm$ SD. The pharmacokinetic and pharmacodynamic variables after the two pretreatments were compared by repeated-measures ANOVA with treatment sequence as a factor or, in the case of $t_{\max}$, using the Wilcoxon signed-rank test. For all variables except $t_{\max}$, 95% confidence intervals were calculated on the mean differences between the placebo and rofecoxib phases. The Pearson correlation coefficient and linear regression analysis were used to investigate possible relationships between the pharmacokinetics of tizanidine and rofecoxib and the caffeine/paraxanthine ratio. All data were analysed using the statistical program Systat for Windows, version 6.0.1 (SPSS Inc., Chicago, IL, USA). The differences were considered statistically significant at $P < 0.05$.

Results

Rofecoxib raised the $C_{\max}$ of tizanidine 6.1-fold (range 4.3–14.7-fold; $P < 0.001$) and its $AUC_{0-\infty}$ 13.6-fold (range 7.4–22.5-fold; $P < 0.001$) compared with the placebo phase (Figure 2, Table 2). The $t_{1/2}$ of tizanidine was increased from 1.6 ± 0.2 h to 3.0 ± 0.6 h ($P < 0.001$) by rofecoxib.

Rofecoxib caused considerable prolongation of the $t_{\max}$ of the tizanidine metabolite M-3 (from 90 min to 300 min; $P = 0.003$) and of its further metabolite M-4 (from 60 min to 300 min; $P = 0.003$) (Figure 3, Table 3). In addition, the $t_{1/2}$ values of M-3 and M-4 were increased by 4.4-fold and 1.6-fold, respectively. Rofecoxib increased the $AUC_{0-\infty}$ of M-3 ($P = 0.01$) and decreased the $C_{\max}$ of M-4 ($P < 0.001$). The tizanidine/M-3 and tizanidine/M-4 ratios in plasma were increased over 10-fold by rofecoxib ($P < 0.001$; Figure 3).

Rofecoxib increased the urinary excretion of tizanidine by 12-fold ($P = 0.04$) and that of M-10 by twofold ($P < 0.001$). However, the excretion of M-4 was decreased by 48% ($P = 0.002$; Figure 4A). Rofecoxib increased by several fold the tizanidine to metabolite (M-3, M-4, M-5, M-8, M-10) ratios in urine (Figure 4B). The renal clearance of tizanidine was not significantly altered by rofecoxib (data not shown).

Tizanidine had a much greater pharmacodynamic effect during the rofecoxib phase than during the pla-

Figure 2

Plasma concentrations (mean $\pm$ SD) of tizanidine after a single oral dose of 4 mg tizanidine during the placebo and rofecoxib (25 mg day⁻¹) phases in nine healthy subjects. $\circ$, placebo phase; $\bullet$, rofecoxib phase. Inset depicts the same data on a semilogarithmic scale

cebo phase (Table 4, Figure 5). During the rofecoxib phase, the mean minimum systolic blood pressure was 97 ± 12 mmHg, which is 20 mmHg lower than during the placebo phase ($P = 0.003$). Similarly, diastolic blood pressure was decreased by tizanidine more during the rofecoxib phase than during the placebo phase ($P = 0.002$). In addition, the effects of tizanidine on subjective drowsiness and overall drug effect were enhanced by rofecoxib ($P < 0.05$, Table 4).

There was no significant difference in the QT-interval or heart rate, recorded using a 12-lead electrocardiogram in the supine position, between the rofecoxib and placebo phases at baseline (data not shown). Two hours after tizanidine ingestion, heart rate was lower during the rofecoxib phase (42 ± 6 beats min⁻¹) than during the placebo phase (47 ± 5 beats min⁻¹) ($P = 0.02$). There was no significant difference between the phases in the heart rate corrected QT-interval (QTc) ($P = 0.15$).

Rofecoxib increased the caffeine/paraxanthine ratio by 2.4-fold (from 1.6 ± 0.7 to 3.7 ± 2.4 ; $P = 0.008$) (Table 1). The AUC of tizanidine, the tizanidine/M-3 AUC ratio and the tizanidine/M-4 AUC ratio correlated well with the caffeine/paraxanthine ratio ($r = 0.78$, $P = 0.014$; $r = 0.78$, $P < 0.001$; and $r = 0.69$, $P = 0.002$, respectively) (Figure 6). During the placebo phase, the tizanidine/metabolite (M-4, M-5 and M-10) Ae ratios correlated significantly with the caffeine/paraxanthine ratio (Table 5). Moreover, the changes in tizanidine/metabolite (M-3, M-4, M-5 and M-9) Ae ratios caused by rofecoxib correlated with the changes in the caffeine/paraxanthine ratio caused by this drug (Table 5).

The concentration of rofecoxib in plasma was approximately 200 ng ml⁻¹ at the time of tizanidine ingestion

Table 2

Pharmacokinetic variables for tizanidine (4 mg) during the placebo and rofecoxib (25 mg day⁻¹) phases in nine healthy subjects

Variable	Placebo phase (control)	Rofecoxib phase	95% CI	P-value
C_{max} (ng ml ⁻¹)	1.7 ± 0.9	10.5 ± 4.0		<0.001
Relative to control (range)		6.1 (4.3–14.7)	4.9, 7.4	
t_{max} (min)	60 (40, 120)	90 (40, 180)		0.74
$t_{1/2}$ (h)	1.6 ± 0.2	3.0 ± 0.6		<0.001
Relative to control (range)		1.90 (1.36–2.70)	1.56, 2.13	
$AUC_{0-\infty}$ (ng h ml ⁻¹)	5.7 ± 3.6	66.7 ± 33.7		<0.001
Relative to control (range)		13.6 (7.4–22.5)	7.9, 15.5	

Data are mean $\pm$ SD or mean with 95% CI; t_{max} data are given as median with range. CI, Confidence interval; C_{max} , maximum plasma concentration; t_{max} , time to reach C_{max} ; $t_{1/2}$, half-life; $AUC_{0-\infty}$, area under plasma concentration–time curve from time 0 to infinity.

Figure 3

Plasma concentrations (mean $\pm$ SD) of tizanidine metabolites M-3 and M-4 (top) and the plasma tizanidine to metabolite concentration ratios (bottom) after a single dose of 4 mg tizanidine during the placebo (○) and rofecoxib (25 mg day⁻¹) (●) phases in nine healthy subjects. The insets depict the same data on a semilogarithmic scale

(Figure 7A). There was a fourfold interindividual variation in the $AUC_{0-\infty}$ and $t_{1/2}$ of rofecoxib and a twofold variation in its C_{max} (Table 1). The trough concentration (predose, C_0) of rofecoxib on day 4 ($r = 0.73$, $P = 0.026$; Figure 7B), the AUC_{0-25} of rofecoxib ($r = 0.80$, $P = 0.01$; Figure 7C) and the $AUC_{0-\infty}$ of rofecoxib ($r = 0.92$, $P = 0.0005$) correlated significantly with the placebo phase caffeine/paraxanthine ratio. The AUC_{0-25} and $AUC_{0-\infty}$ of rofecoxib also correlated with the placebo phase $AUC_{0-\infty}$ of tizanidine ($r = 0.62$, $P = 0.077$, and $r = 0.70$, $P = 0.037$).

The subjects complained more about dry mouth and tiredness after tizanidine ingestion during the rofecoxib phase than during the placebo phase. During the first 12 h, the urine volume was 764 ± 393 ml in the rofecoxib phase and 1226 ± 360 ml in the placebo phase

($P = 0.004$), but there was no significant difference between the phases in the urine volume excreted between 12 and 24 h.

Discussion

Our study using tizanidine and caffeine as probe substrates of CYP1A2 indicates that rofecoxib, at a normal daily dose of 25 mg, is a potent inhibitor of CYP1A2 activity. Rofecoxib showed a particularly strong pharmacokinetic interaction with tizanidine, which has potentially hazardous consequences. The $AUC_{0-\infty}$ of tizanidine was increased in each of the nine subjects by an average of nearly 14-fold, the greatest increase being more than 22-fold. Thus, it is most probable that the cardiovascular and central nervous system adverse reactions (e.g. hypotension, bradycardia, somnolence),

Table 3

Pharmacokinetic variables for tizanidine metabolites 3, 4 and 5 during the placebo and rofecoxib (25 mg day⁻¹) phases in nine healthy subjects

Variable	Placebo phase (control)	Rofecoxib phase	95% CI	P-value
<i>Metabolite 3</i>				
$C_{\max}$ (U ml ⁻¹)	0.45 ± 0.11	0.38 ± 0.81		0.14
Relative to control (range)		0.84 (0.50–1.50)	0.53, 1.11	
$t_{\max}$ (min)	90 (60, 180)	300 (180, 1400)		0.003
$t_{1/2}$ (h)*	4.3 ± 1.5	17.5 ± 8.1		0.002
Relative to control (range)		4.40 (2.90–6.80)	2.56, 5.65	
AUC _{0–24} (U h ml ⁻¹)	3.1 ± 1.1	6.4 ± 1.4		<0.001
Relative to control (range)		2.06 (1.40–3.90)	1.77, 2.32	
AUC _{0–∞} (U h ml ⁻¹)*	3.0 ± 1.3	12.3 ± 9.1		0.011
Relative to control (range)		4.10 (2.70–8.60)	1.73, 6.47	
<i>Metabolite 4</i>				
$C_{\max}$ (U ml ⁻¹)	1.3 ± 0.1	0.64 ± 0.13		<0.001
Relative to control (range)		0.51 (0.38–0.70)	0.42, 0.64	
$t_{\max}$ (min)	60 (40, 240)	300 (240, 420)		0.003
$t_{1/2}$ (h)	4.7 ± 0.7	8.0 ± 4.0		0.016
Relative to control (range)		1.60 (1.20–2.80)	1.15, 2.23	
AUC _{0–24} (U h ml ⁻¹)	10.0 ± 1.3	7.4 ± 1.1		<0.001
Relative to control (range)		0.75 (0.59–0.86)	0.65, 0.85	
AUC _{0–∞} (U h ml ⁻¹)	10.4 ± 1.6	9.1 ± 3.2		0.16
Relative to control (range)		0.87 (0.69–1.40)	0.71, 1.05	
<i>Metabolite 5</i>				
$C_{\max}$ (U ml ⁻¹)	0.10 ± 0.06	0.17 ± 0.07		0.09
Relative to control (range)		1.70 (0.22–5.60)	0.9, 2.5	
$t_{\max}$ (min)	60 (40, 240)	180 (60, 420)		0.046

Data are mean ± SD or mean with 95% CI; $t_{\max}$ data are given as median with range. CI, Confidence interval; $C_{\max}$, maximum plasma concentration; $t_{\max}$, time to reach $C_{\max}$; $t_{1/2}$, half-life; AUC_{0–24}, area under plasma concentration–time curve from time 0–24 h; AUC_{0–∞}, area under plasma concentration–time curve from time 0 to infinity. *Data from eight subjects only are shown, because the $t_{1/2}$ and AUC_{0–∞} could not be determined for subject 8 (the individual with the lowest CYP1A2 activity), because M-3 concentration was rising up to the last time point (24 h).

observed in several patients after the concomitant intake of rofecoxib and tizanidine [1, 2], were caused by this pharmacokinetic interaction. Until now, the mechanism of this suspected interaction was unknown. Furthermore, our results indicate that inhibition of several CYP1A2 mediated routes of tizanidine metabolism by rofecoxib (and/or its metabolites) explains this pharmacokinetic interaction.

CYP1A2 makes a large contribution to the elimination of tizanidine [8, 10, 11], the major metabolites of which are M-3, M-4 and M-10 [9]. In the present study, M-3 and M-4 were detected in plasma, and M-3, M-4, M-5, M-9 and M-10 in urine. Owing to lack of reference compounds, the metabolite concentrations could be determined only in arbitrary units. However, this does not compromise the estimation of the correlation

between CYP1A2 activity and the different metabolite ratios of tizanidine, or of the effect of rofecoxib on the formation of these metabolites.

In addition to increasing greatly the plasma concentrations of tizanidine, rofecoxib increased by several fold the tizanidine/metabolite ratios and by 2.4-fold the caffeine/paraxanthine ratio. The latter, an established indicator of CYP1A2 activity [12–14], correlated with the tizanidine/metabolite ratios in plasma and urine (Figure 6 and Table 5), suggesting that CYP1A2 is involved in the formation of all tizanidine metabolites. Moreover, the increases in the plasma concentration and urinary excretion of tizanidine and the tizanidine/metabolite (M-3, M-4, M-5 and M-9) ratios caused by rofecoxib correlated well with the increase in the caffeine/paraxanthine ratio. These findings indicate that rofe-

Figure 4

Cumulative excretion of tizanidine and its metabolites (mean ± SD) into urine (A) and the tizanidine to metabolite 24 h excretion ratios in urine (B) after a single dose of 4 mg tizanidine during the placebo (○ and white bars) and rofecoxib 25 mg day⁻¹ (● and black bars) phases. **P* < 0.05; ***P* < 0.01; ****P* < 0.001 vs. placebo

coxib is a potent inhibitor of CYP1A2 *in vivo*, and that the rofecoxib–tizanidine interaction is mediated by inhibition of the CYP1A2-mediated metabolism of tizanidine.

Although rofecoxib inhibited the formation of M-3 from tizanidine, the further metabolism of M-3 (a precursor of M-4) was also inhibited by rofecoxib. Consequently, the total AUC and *t*_{1/2} of M-3 were increased markedly, whereas the AUC of M-4 decreased slightly. M-4 can also be formed from M-5, the half-life of which seems to be relatively short. Therefore, the half-life of M-4 was not formation rate-limited by the long half-life of M-3. On the other hand, rofecoxib increased the excretion of M-10 into urine during the first 12 h after tizanidine intake, and the increase in the tizanidine/M-10 ratio by rofecoxib treatment did not correlate significantly with the increase in the caffeine/paraxanthine ratio. These findings suggest that, in addition to CYP1A2, other enzymes may be involved in the formation of M-10.

Recently, the effect of two established inhibitors of CYP1A2, fluvoxamine (100 mg given once daily for

4 days) and ciprofloxacin (500 mg twice daily for 3 days), on the pharmacokinetics and pharmacodynamics of tizanidine and on the caffeine test has been studied [8, 11]. The increase in tizanidine AUC_{0-∞} by rofecoxib (14-fold) in the present study was slightly greater than that seen by ciprofloxacin (10-fold) [8] but smaller than by fluvoxamine (33-fold) [11]. The *C*_{max} of tizanidine was increased by rofecoxib (sixfold) to the same extent or less than by ciprofloxacin (sevenfold) and fluvoxamine (12-fold). On the other hand, the half-life of tizanidine was nearly doubled by rofecoxib (from 1.6 to 3.0 h), only slightly prolonged by ciprofloxacin (from 1.5 to 1.8 h) and nearly tripled by fluvoxamine (from 1.5 to 4.3 h). In these three studies, a single 4-mg dose of tizanidine was given 1 h after the other drug in order to allow the latter to dissolve and undergo significant absorption. However, the elimination half-lives of rofecoxib (22 h), ciprofloxacin (3–4 h) and fluvoxamine (7–60 h) differ considerably [20, 21]. Therefore, ciprofloxacin seems to have inhibited mainly the presystemic metabolism (reflected as an increased *C*_{max}) of tizanidine, with a minimal effect during the elimination phase [8],

Table 4Pharmacodynamic effects of tizanidine (4 mg) during the placebo and rofecoxib (25 mg day⁻¹) phases in nine healthy subjects

Variable	Placebo phase (control)	Rofecoxib phase	Difference between phases, 95% CI	P-value
<i>Systolic blood pressure</i>				
Baseline (mmHg)	128 ± 10	132 ± 4	3.9 (–2.6, 10.3)	0.21
Minimum (mmHg)	117 ± 10	97 ± 12	–20 (–32, –9)	0.004
AUC _{0–12} (mmHg h)	1570 ± 100	1410 ± 120	–153 (–211, –94)	<0.001
<i>Diastolic blood pressure</i>				
Baseline (mmHg)	76 ± 6	77 ± 7	0.9 (–3.9, 5.8)	0.78
Minimum (mmHg)	65 ± 8	53 ± 7	–12 (–18, –5)	0.003
AUC _{0–12} (mmHg h)	880 ± 72	770 ± 77	–107 (–142, –72)	<0.001
<i>Heart rate</i>				
Baseline (beats min ⁻¹)	59 ± 7	56 ± 7	–3 (–7, 1)	0.08
Minimum (beats min ⁻¹)	50 ± 5	49 ± 8	–1 (–5, 3)	0.50
AUC _{0–12} (beats min ⁻¹ h)	696 ± 86	682 ± 89	–14 (–50, 22)	0.312
<i>VAS: drowsiness</i>				
Baseline (mm)	30 ± 19	33 ± 20	3 (–10, 16)	0.95
Maximum (mm)	41 ± 19	71 ± 22	30 (13, 47)	0.007
AUC _{0–12} (mm h)	215 ± 138	385 ± 185	169 (82, 255)	0.005
<i>VAS: drug effect</i>				
Baseline (mm)	1 ± 3	2 ± 3	1 (–2, 4)	0.47
Maximum (mm)	14 ± 13	41 ± 22	27 (12, 43)	0.006
AUC _{0–12} (mm h)	36 ± 39	152 ± 120	116 (35, 196)	0.016
<i>DSST</i>				
Baseline (symbols per 2 min)	104 ± 10	105 ± 13	0.2 (–5.9, 6.3)	0.60
Minimum (symbols per 2 min)	96 ± 11	86 ± 18	–11 (–22, 1)	0.07
AUC(0,12) (symbols per 2 min h)	1255 ± 156	1199 ± 174	–56 (–107, 4)	0.06

The maximum effects (maximum or minimum value after tizanidine) and AUC_{0–12} are presented for systolic and diastolic blood pressure, heart rate and for the psychomotor tests. Data are mean ± SD or mean with 95% CI. AUC_{0–12}, area under effect–time curve from time 0–12 h; VAS, visual analogue scale; DSST, digit symbol substitution test.

whereas rofecoxib affected both presystemic and systemic metabolism ($t_{1/2}$, AUC) of tizanidine. Fluvoxamine, which is a highly potent inhibitor of CYP1A2 [22] and has a long half-life, had the greatest effect on both the presystemic and systemic elimination of tizanidine [11].

In general, the cardiovascular and central nervous system effects of tizanidine correlate well with its plasma concentration [11]. Accordingly, rofecoxib caused substantial potentiation and prolongation of both the hypotensive and sedative effects of tizanidine. The hypotensive effect of tizanidine was increased by rofecoxib, although treatment with the latter itself, in line with previous findings [23, 24], also tended to increase blood pressure slightly. The maximum systolic hypotensive response to tizanidine during rofecoxib (–35 mmHg) was much greater than during placebo (–11 mmHg). In the present study, rofecoxib also slightly increased the negative chronotropic effect of tizanidine, as shown by the occurrence of bradycardia

(42 beats min⁻¹), evident in the supine position at 2 h postdose during the rofecoxib phase.

Cases of symptomatic hypotension, bradycardia and somnolence have been observed (one with a fatal outcome) during coadministration of rofecoxib and tizanidine [1, 2], demonstrating the potential hazards of this interaction. Similar exaggerated pharmacodynamic effects of tizanidine were seen in our healthy subjects after ingestion of tizanidine during the rofecoxib phase. In one case report, QT-prolongation was also observed during the combination [2]. However, we found no evidence of QTc-prolongation after tizanidine intake during either the rofecoxib or placebo phases. Urine volume during the first 12 h after tizanidine ingestion was markedly lower during the rofecoxib phase than during the placebo phase, even though the hypotensive subjects in particular were prompted to drink water. Thus, concomitant administration of rofecoxib and tizanidine seems to affect renal function adversely, and may increase the

Figure 5

Mean $\pm$ SD systolic and diastolic blood pressures (RR) and recordings of subjective drowsiness and drug effect (VAS, visual analogue scale) after a single dose of 4 mg tizanidine during the placebo (O) and rofecoxib 25 mg day⁻¹ (●) phases in nine healthy subjects

risk of fluid accumulation and oedema. This renal effect was probably mainly due to the hypotension caused by the high plasma concentrations of tizanidine. However, rofecoxib may also decrease renal function and cause fluid retention, which may have contributed to the decreased urine excretion [23, 24].

Rofecoxib, in daily doses of 12.5, 25 and 50 mg, increased the AUC of R(+)-warfarin by 1.27-, 1.38- and 1.40-fold [25] and the AUC of theophylline by 1.4-, 1.5- and 1.6-fold, respectively [6]. The product information on rofecoxib states that 'these data suggest that rofecoxib may produce a modest inhibition of cytochrome P450 (CYP) 1A2' [4]. However, the findings of the present study with caffeine and tizanidine indicate that rofecoxib

is a potent inhibitor of CYP1A2. It is reasonable to assume that a larger daily dose of rofecoxib could have an even greater effect on tizanidine pharmacokinetics. Other CYP1A2 substrates include several important drugs, such as clozapine, olanzapine, riluzole, tacrine, zolmitriptan, lidocaine, ropivacaine and melatonin [26–35]. It is likely that rofecoxib, in a dose-dependent manner, will also increase their plasma concentrations and enhance their pharmacological actions. The effect of rofecoxib may depend on the pharmacokinetic properties of the substrate drug, e.g. the extent of first-pass metabolism. However, no studies seem to have been carried out to determine whether treatment with rofecoxib has led to adverse events in patients taking CYP1A2 substrate drugs.

Figure 6

Relationships between the $AUC_{0-\infty}$ tizanidine, and the tizanidine/metabolite (M-3 or M-4) AUC_{0-24} ratios and the caffeine/paraxanthine ratio. ○, Placebo phase; ●, rofecoxib phase. Correlations were tested using linear regression analysis

Table 5

Relationships between the tizanidine/metabolite urine Ae ratios and the caffeine/paraxanthine plasma concentration ratio during the placebo phase, and between their changes caused by rofecoxib

Variable	Caffeine/paraxanthine ratio			
	Values during placebo		Changes caused by rofecoxib	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
<i>Urine Ae ratio</i>				
Tizanidine/M-3	0.65	0.06	0.90	< 0.001
Tizanidine/M-4	0.79	0.011	0.73	0.025
Tizanidine/M-5	0.76	0.018	0.92	< 0.001
Tizanidine/M-9	0.58	0.10	0.86	0.003
Tizanidine/M-10	0.79	0.011	0.56	0.12

r, Pearson's correlation coefficient; Ae, amount excreted in urine within 24 h.

Rofecoxib itself is extensively metabolized via complex oxidative, reductive and reverse reductive pathways and it was suggested to have a relatively low propensity to interact with drugs that are metabolized by CYP enzymes [4, 36]. According to the product information, 'cytochrome P450 plays a minor role in metabolism of rofecoxib' [4, 36]. However, CYP1A2 has been found to catalyse the oxidative metabolism of rofecoxib *in vitro* [36]. A fourfold interindividual variability in the AUC of rofecoxib was observed in our study, which was carried out in a homogeneous group of young healthy subjects. The AUC of rofecoxib correlated well with the caffeine/paraxanthine ratio (Figure 7), which suggests that CYP1A2 has a significant role in the elimination of the drug. A role for CYP1A2 in rofecoxib metabolism is further supported by the previous observations that the pharmacokinetics of the drug are nonlinear, being best described by auto-inhibition of its own CYP1A2-mediated metabolism [4, 37]. Accordingly, interindividual variability in CYP1A2 activity may contribute to the risk of concentration-dependent adverse effects of rofecoxib, such as oedema, sodium retention and, theoretically, even an increased risk of myocardial infarction [3, 4, 23, 24]. Thus, coadministration of other CYP1A2 inhibitors, such as oral contraceptives, ciprofloxacin or fluvoxamine [8, 11], might lead to an accumulation of rofecoxib during daily administration and an increased risk of adverse events.

In conclusion, rofecoxib is a potent inhibitor of CYP1A2 and it greatly increases the plasma concentra-

Figure 7

Plasma concentrations of rofecoxib (mean $\pm$ SD) on day 4 of treatment with 25 mg rofecoxib once daily in nine healthy subjects (A). Time 0 refers to the administration of rofecoxib, which was given 1 h before the administration of tizanidine. Relationship between the plasma caffeine/paraxanthine ratio during the placebo phase and the C_0 of rofecoxib (B) and between the plasma caffeine/paraxanthine ratio during the placebo phase and the AUC_{0-25} of rofecoxib (C) are also shown. Correlations were tested using linear regression analysis

tions and adverse effects of tizanidine, a CYP1A2 substrate undergoing substantial presystemic metabolism. Therefore, coadministration of rofecoxib and tizanidine is best avoided. The findings also suggest that CYP1A2 plays a significant role in the elimination of rofecoxib and raise concerns about the potential risks of interactions between the drug and other CYP1A2 substrates and inhibitors.

Conflict of interest

None declared.

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