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Effects of the Antifungals Voriconazole and Fluconazole on the Pharmacokinetics of *S*-(+)- and *R*-(−)-Ibuprofen

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Our objective was to study the effects of the antifungals voriconazole and fluconazole on the pharmacokinetics of *S*-(+)- and *R*-(−)-ibuprofen. Twelve healthy male volunteers took a single oral dose of 400 mg racemic ibuprofen in a randomized order either alone, after ingestion of voriconazole at 400 mg twice daily on the first day and 200 mg twice daily on the second day, or after ingestion of fluconazole at 400 mg on the first day and 200 mg on the second day. Ibuprofen was ingested 1 h after administration of the last dose of voriconazole or fluconazole. Plasma concentrations of *S*-(+)- and *R*-(−)-ibuprofen were measured for up to 24 h. In the voriconazole phase, the mean area under the plasma concentration-time curve (AUC) of *S*-(+)-ibuprofen was 205% ($P < 0.001$) of the respective control value and the mean peak plasma concentration (C_{max}) was 122% ($P < 0.01$) of the respective control value. The mean elimination half-life ($t_{1/2}$) was prolonged from 2.4 to 3.2 h ($P < 0.01$) by voriconazole. In the fluconazole phase, the mean AUC of *S*-(+)-ibuprofen was 183% of the control value ($P < 0.001$) and its mean C_{max} was 116% of the control value ($P < 0.05$). The mean $t_{1/2}$ of *S*-(+)-ibuprofen was prolonged from 2.4 to 3.1 h ($P < 0.05$) by fluconazole. The geometric mean *S*-(+)-ibuprofen AUC ratios in the voriconazole and fluconazole phases were 2.01 (90% confidence interval [CI], 1.80 to 2.22) and 1.82 (90% CI, 1.72 to 1.91), respectively, i.e., above the bioequivalence acceptance upper limit of 1.25. Voriconazole and fluconazole had only weak effects on the pharmacokinetics of *R*-(−)-ibuprofen. In conclusion, voriconazole and fluconazole increased the levels of exposure to *S*-(+)-ibuprofen 2- and 1.8-fold, respectively. This was likely caused by inhibition of the cytochrome P450 2C9-mediated metabolism of *S*-(+)-ibuprofen. A reduction of the ibuprofen dosage should be considered when ibuprofen is coadministered with voriconazole or fluconazole, especially when the initial ibuprofen dose is high.

Voriconazole is a novel triazole antifungal agent, available as oral and intravenous formulations, with potent activity against a broad spectrum of clinically significant pathogens, including *Aspergillus*, *Cryptococcus*, and *Candida* species (5, 8, 27). Voriconazole is metabolized by the cytochrome P450 (CYP) enzyme system, mainly by the polymorphic enzyme CYP2C19 and to a lesser extent by the polymorphic enzymes CYP2C9 and CYP3A4 (13; G. Mikus, M. Drzevinska, V. Schoevel, J. Burhenne, K. D. Riedel, T. Thomsen, M. M. Hoffmann, J. Weis, J. Rengelshausen, and W. E. Haefeli, Abstr. 7th Congr. Eur. Assoc. Clin. Pharmacol. Ther., abstr. 418, 2005). In vivo studies have shown that addition of voriconazole at 300 mg twice daily to a 30-mg oral dose of warfarin in healthy volunteers resulted in a doubling of the prothrombin time from 8.4 to 16.6 s (28), probably due to inhibition of CYP2C9. In addition, coadministration of voriconazole has significantly increased the area under the plasma concentration-time curve (AUC) of phenytoin, a substrate of CYP2C9 (30); the AUC of omeprazole, a substrate of CYP2C19 (http://www.emea.eu.int./humandocs/Humans/EPAR/vfend/vfend.htm); and the AUC of cyclosporine, which is metabolized by CYP3A4 (31).

Fluconazole is another azole antifungal agent and a well-documented potent inhibitor of CYP2C9-catalyzed reactions both in vitro (3, 20) and in vivo (4, 18) and a weaker inhibitor of CYP3A4-catalyzed reactions (23). It has also been found to inhibit CYP2C19-catalyzed reactions both in vitro (34) and in vivo (17). However, there are few data on the possible interactions between fluconazole and nonsteroidal anti-inflammatory drugs (NSAIDs). Treatment with fluconazole significantly increased the AUC of the CYP2C9 substrate celecoxib (7), and recently, it has been shown that coadministration of fluconazole and a novel cyclooxygenase 2 (COX-2)-selective inhibitor, lumiracoxib, metabolized by CYP2C9, caused a small (18%) increase in the mean AUC of lumiracoxib (32).

Ibuprofen is a chiral NSAID widely used worldwide, and it is available as an over-the-counter remedy in many countries. Ibuprofen is a nonselective inhibitor of COX-1 and COX-2. Most preparations contain the racemic mixture of *R*-(−)- and *S*-(+)-ibuprofen, with the *S*-(+) enantiomer of ibuprofen possessing the majority of pharmacological activity (1, 9). After administration of racemic ibuprofen, about 60% of *R*-(−)-ibuprofen is inverted to the *S*-(+)-enantiomer in the human body, but there is no measurable inversion in the other direction (2, 16). The major pathway for ibuprofen elimination is

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oxidative metabolism to hydroxylated metabolites. In vitro studies have indicated the stereoselectivity of ibuprofen hydroxylation, with CYP2C9 being the main enzyme involved in the hydroxylation of *S*-(+)-ibuprofen and CYP2C8 being the main enzyme involved in the hydroxylation of *R*-(−)-ibuprofen (12).

Both CYP2C9 and CYP2C8 exhibit genetic polymorphism, with interethnic differences in the frequency of mutant alleles. The frequencies of mutant alleles *CYP2C9*2* and *CYP2C9*3* have been reported to range from 8 to 19% and from 3.3 to 16.2% in Caucasian populations, respectively (35), and the mutant allele *CYP2C8*3* has an 8.9 to 13% allelic frequency in Caucasians (6, 24). It has been found that the mean *S*-(+)-ibuprofen clearance was significantly decreased in individuals who are hetero- or homozygous for *CYP2C9*3* compared with that in individuals with the wild-type genotype, whereas the *CYP2C9*2* variant had no significant effect on *S*-(+)-ibuprofen clearance (19). In addition, CYP2C9 polymorphism had no significant influence on *R*-(−)-ibuprofen metabolism.

Although ibuprofen is considered a well-tolerated drug, it is not free of adverse events. Gastritis, peptic ulcer, minor or major upper intestinal bleeding, analgesic nephropathy, and fluid retention are typical concentration-dependent adverse effects of ibuprofen. The coadministration of voriconazole or fluconazole and ibuprofen may lead to increased levels of ibuprofen and increase the risk for ibuprofen-related adverse events. However, no clinical studies have addressed the effect of either voriconazole or fluconazole on the pharmacokinetics of ibuprofen. Therefore, the aim of this study was to clarify the possible interaction between these azole antifungals and ibuprofen.

MATERIALS AND METHODS

Subjects and ethics. Twelve male volunteers (age range, 19 to 23 years; body mass index range, 20 to 26 kg/m^2) participated in this study. The subjects were ascertained to be in good health by medical history, physical examination, and standard hematological and clinical chemistry tests. All subjects were nonsmokers and used no concomitant medications. The subjects received both verbal and written information on the study, and written informed consent was obtained. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland.

Protocol. This study had an open, randomized, crossover design with three phases, with a washout period of 2 weeks between the phases. In the control phase, the subjects received a single 400-mg oral dose of racemic ibuprofen (Burana; Orion, Helsinki, Finland) with no pretreatment. In the second and third phases, the subjects ingested as pretreatment either fluconazole (Fluconazol ratiopharm; Merkle GmbH, Blauburen, Germany) at 400 mg as a single daily dose on the first day and 200 mg on the second day or voriconazole (Vfend; Pfizer, Sandwich, United Kingdom) at 400 mg twice daily on the first day and 200 mg twice daily on the second day. The oral dose of 400 mg racemic ibuprofen was given 1 h after the last dose of voriconazole or fluconazole at 9 a.m. with 150 ml of water.

To determine the plasma concentrations of *S*-(+)- and *R*-(−)-ibuprofen, a 10-ml blood sample was drawn via a venous forearm cannula before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 h after the ibuprofen dose. The plasma was separated and stored at −70°C until the *S*-(+)- and *R*-(−)-ibuprofen concentrations were analyzed by high-performance liquid chromatography (HPLC).

During all phases, the subjects fasted overnight before administration of ibuprofen and continued fasting until a standardized lunch was served 4 h after ibuprofen ingestion. The subjects did not use any other medication or natural products for 14 days before and during the study and no drug known to cause enzyme induction or inhibition for a period of 30 days prior the study. Caffeine, grapefruit juice, and alcohol-containing beverages were not allowed during the study. All fluconazole and voriconazole doses except for the last doses were self-administered by the subjects; the last doses were administered by the study personnel. The intake of the premedication by the subjects was verified by having the subjects send a short message to the investigators by mobile phone right after they took each dose of voriconazole or fluconazole. Compliance was also ascertained by quantifying plasma voriconazole and fluconazole levels on the study days before the last administration of these drugs.

Genotyping. Genotyping for *CYP2C9*2*, *CYP2C9*3*, and *CYP2C8*3* was performed by a 5′-nuclease assay. For *CYP2C9*2*, primers CAA TGG AAA GAA ATG GAA GGA GAT and AAG ATA GTA GTC CAG TAA GGT CAG TGA TAT G (Cybergene AB, Novum Research Park, Sweden) were used with minor grove binding dark quencher MGB probes VIC-TTG AAC AC**A** GTC CTC A-MGB and FAM-TTG AAC AC**G** GTC CTC-MGB (Applied Biosystems, Cheshire, United Kingdom), where boldface indicates the specific places of the probes and VIC and FAM are the fluorescent dyes used to differentiate each probe. For *CYP2C9*3*, primers CAG GAA GAG ATT GAA CGT GTG ATT and CTA TGA ATT TGG GGA CTT CGA AA and probes FAM-AGA TAC **C**TT GAC CTT CT-MGB and VIC-AGA GAT AC**A** TTG ACC TTC-MGB were used. For *CYP2C8*3*, the primers were ATG TCC ACT ACT TCT CCT CAC TTC TG and AAA GTG GCC AGG GTC AAA GA, and the probes were FAM-TG ATG ACA GAG AAT TT and VIC-ATG ATG ACA AAG AAT TT. Each reaction used the primers at a final concentration of 450 nM and the probes at a final concentration of 75 nM. The reaction volume used was 25 μl, and the PCR setting was 50°C for 10 min and 95°C for 10 min, followed by 35 times cycling of 95°C for 15 s, after which the PCR setting was 60°C for 1 min. Each sample was run in duplicate.

Bioanalysis of ibuprofen. The plasma concentrations of *R*-(−)- and *S*-(+)-ibuprofen were determined by HPLC with UV detection at 220 nm (22, 26). Briefly, *R*-(−)- and *S*-(+)-ibuprofen and the internal standard, fenoprofen, were extracted from plasma with ethyl acetate. After evaporation, the residue was reconstituted with the mobile phase and the supernatant was used for injection. The mobile phase was a mixture of 2-propanol (0.25%) and 50 mM sodium hydrogen phosphate buffer (pH 7.0; 99.75%). Chromatographic analysis was performed on a Chiral AGP analytical column (150 by 4 mm [inner diameter]; Chrom Tech Ltd., Congleton, United Kingdom) protected with a Chiral AGP Guard column (10 by 4 mm [inner diameter]; Chrom Tech Ltd.). Because a voriconazole metabolite interfered with the HPLC analysis of *R*-(−)-ibuprofen, the concentrations of *R*-(−)-ibuprofen during the voriconazole phase were quantified with a liquid chromatography-tandem mass spectrometry (LC/MS/MS) system (MDS SCIEX; Q Trap LC/MS/MS system; Applied BioSystems, Foster City, CA) by using a mixture of 2-propanol (0.25%) and 10 mM ammonium acetate (pH 6.5; 99.75%) as the mobile phase. The mass spectrometer was operated in the atmospheric pressure chemical ionization mode with negative ion detection. The ion transitions monitored were a mass-to-charge ratio (*m*/*z*) 204.9 to *m*/*z* 161.2 for *R*-(−)- and *S*-(+)-ibuprofen and *m*/*z* 241.0 to *m*/*z* 93.1 for the internal standard, fenoprofen. These transitions represent the product ion of the $[M\text{-}H]^-$ ion. The day-to-day coefficients of variation (CVs) were less than 12% for both enantiomers at concentrations of 500 ng/ml, 5,000 ng/ml, and 25,000 ng/ml ($n = 11$ for the HPLC method; $n = 5$ for the LC/MS/MS method). The limit of quantification for *R*-(−)- and *S*-(+)-ibuprofen was 250 ng/ml. The HPLC and LC/MS/MS methods used gave identical results at the relevant concentrations for both enantiomers during all other study phases except during the voriconazole phase for *R*-(−)-ibuprofen.

Bioanalysis of voriconazole and fluconazole. After a solid-phase extraction of voriconazole from plasma, its concentration was determined by HPLC with UV detection at 255 nm by using a fluconazole analog (UK 54373) as the internal standard (10, 25). The limit of voriconazole quantification was 0.05 mg/liter, and the CV was less than 4% at the relevant concentrations (0.05 mg/liter, 1.0 mg/liter, and 10.0 mg/liter; $n = 7$). The plasma fluconazole concentrations were determined after a solid-phase extraction by HPLC with UV detection at 210 nm by using UK 54373 as the internal standard (15). The limit of fluconazole quantification was 0.2 mg/liter. The CV was less than 2% at the relevant concentrations (3 mg/liter and18 mg/liter; $n = 6$ to 7).

Data analysis. The pharmacokinetic parameters for *S*-(+)- and *R*-(−)-ibuprofen were calculated by standard noncompartmental methods (WinNonlin; Pharsight Co., Mountain View, CA). The maximum plasma concentration (C_{max}) and the time to C_{max} (T_{max}) for each subject were derived directly from the plasma concentration data. The AUC was calculated from time zero to infinity by use of the linear trapezoidal rule. The elimination half-life ($t_{1/2}$) was calculated by least-squares regression analysis of the terminal linear part of the log concentration-time curve. The absolute changes in the pharmacokinetic parameters were tested by use of the analysis of variance model for repeated measurements (SPSS 11.0 for Windows 2001; SPSS Inc., Chicago, IL), and Tukey's test was used for post-hoc testing. T_{max} was analyzed by Friedman's test, and the Wilcoxon signed rank test was used for pairwise comparisons. The correlation between the

voriconazole and the fluconazole trough concentrations and the change in the *S*-(+)-ibuprofen AUC were tested by the Pearson correlation test when the data were normally distributed, and the Spearman rank test was used for nonnormally distributed data. The chosen statistical significance level was a P value of <0.05. The results are presented as means ± standard deviations in the text and Table 1 and for clarity are presented as the standard error of the mean in Fig. 1. For T_{max}, the median with the range is shown. The percent differences between treatments were calculated within subjects. Since pharmacokinetic drug interactions can also be assessed statistically by the same methods that are standard for investigation of bioequivalence, we calculated the 90% confidence intervals (CIs) for treatment ratios (coadministration/ibuprofen alone) using log-transformed values of AUC and C_{max}. Bioequivalence (i.e., the lack of an interaction) was concluded if the 90% CI of the geometric mean ratios for both C_{max} and AUC were within the acceptance limit of 0.8 to 1.25.

RESULTS

All subjects completed the study according to the protocol. Four of the 12 subjects had visual disturbances during the voriconazole pretreatment. No other clinically relevant adverse events were recorded during the study. Two subjects had the **1/*3* genotype for *CYP2C9* and were homozygous wild type (**1/*1*) for *CYP2C8*. One subject had the **1/*3* genotype for *CYP2C8* and was homozygous wild-type (**1/*1*) for CYP2C9. All the others were homozygous wild type for both *CYP2C9* and *CYP2C8*. The mean trough concentrations of voriconazole and fluconazole were 1.3 μg/ml and 4.2 μg/ml, respectively, just before the fourth dose of voriconazole and the second dose of fluconazole. The variations in voriconazole and fluconazole trough concentrations between the subjects were about 13-fold (range, 0.3 to 3.9 μg/ml) and 2-fold (range, 3.0 to 5.9 μg/ml), respectively.

***S*-(+)-Ibuprofen pharmacokinetics.** Voriconazole significantly increased the plasma *S*-(+)-ibuprofen concentrations (Fig. 1a). The AUC of *S*-(+)-ibuprofen was increased by 105% ($P < 0.001$) compared with that in the control phase (Table 1). Importantly, the increase was evident in all subjects (Fig. 2a). Similarly, the mean C_{max} of *S*-(+)-ibuprofen was 22% higher ($P < 0.01$) and there was a prolongation of the *S*-(+)-ibuprofen elimination half-life by 43% ($P < 0.01$) compared with the values in the control phase (Table 1; Fig. 2b and c). In addition, a significant correlation was seen between the voriconazole trough plasma concentration and the increase in the *S*-(+)-ibuprofen AUC (Spearman $r = 0.82$; $P < 0.01$). No significant difference in T_{max} between the voriconazole phase and the control phase was noticed.

Fluconazole produced a mean 83% increase in the AUC of *S*-(+)-ibuprofen ($P < 0.001$); the increase was evident in all subjects (Table 1; Fig. 1a and 2a). There was a significant positive correlation between the fluconazole trough concentration and the increase in the AUC of *S*-(+)-ibuprofen (Pearson $r = 0.76$; $P < 0.01$). The C_{max} of *S*-(+)-ibuprofen was 16% higher ($P < 0.05$) and the T_{max} of *S*-(+)-ibuprofen was significantly longer ($P < 0.05$) in the fluconazole phase compared with the values in the control phase (Table 1; Fig. 2b). In addition, there was a significant prolongation of the elimination $t_{1/2}$ of *S*-(+)-ibuprofen (Table 1; Fig. 2c) by 34% ($P < 0.05$).

***R*-(−)-Ibuprofen pharmacokinetics.** The AUC of *R*-(−)-ibuprofen was increased by 20% ($P < 0.05$) in the voriconazole phase compared with that in the control phase (Table 1; Fig. 1b and 3a), whereas the elimination $t_{1/2}$ was slightly shortened by 7% ($P < 0.01$) (Table 1). The C_{max} and T_{max} of *R*-(−)-ibuprofen were unaffected by voriconazole pretreatment (Table 1; Fig. 3b). Fluconazole had no significant effects on the AUC, C_{max}, elimination $t_{1/2}$, or T_{max} of *R*-(−)-ibuprofen (Table 1; Fig. 3).

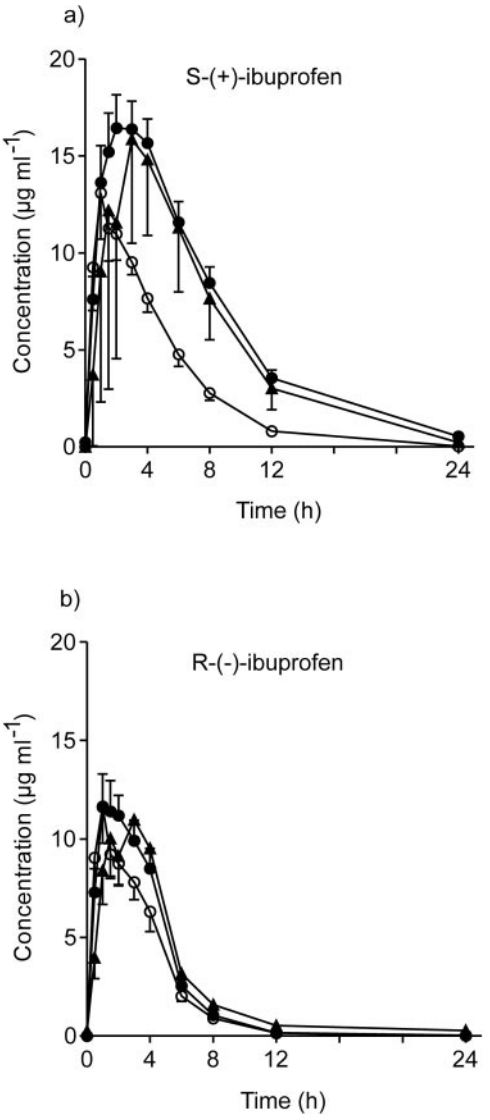

FIG. 1. Mean plasma concentrations (standard errors of the means are indicated by error bars) of *S*-(+)-ibuprofen (a) and *R*-(−)-ibuprofen (b) after the administration of a single 400-mg dose of racemic ibuprofen in 12 healthy male subjects in the control phase (open circles) and after pretreatment with fluconazole (triangles) and after pretreatment with voriconazole (solid circles).

Bioequivalence testing. Geometric mean ratios and 90% CIs are given in Table 1. The geometric mean *S*-(+)-ibuprofen AUC ratios in the voriconazole and fluconazole phases were 2.01 (90% CI, 1.80 to 2.22) and 1.82 (90% CI, 1.72 to 1.91), respectively, i.e., above the bioequivalence acceptance upper limit of 1.25. Also, the 90% CI for the geometric mean ratio of the *S*-(+)-ibuprofen C_{max} was outside the bioequivalence acceptance range for voriconazole but not that for fluconazole. For *R*-(−)-ibuprofen, the only significant finding was that the AUC was outside the acceptance range after pretreatment with voriconazole.

TABLE 1. Pharmacokinetic parameters of *S*-(+)- and *R*-(−)-ibuprofen in 12 subjects after administration of a single oral dose of 400 mg of ibuprofen (racemic) without pretreatment (control), after pretreatment with voriconazole, and after pretreatment with fluconazole[a]

Parameter	Value for:		Ratio of geometric mean (90% CI) for Vori/control	Value for Fluco	Ratio of geometric mean (90% CI) for Fluco/control
	Control	Vori			
S-(+)-Ibuprofen					
AUC ($\mu g \cdot h\ ml^{-1}$)	67.4 ± 16.2	137.2 ± 42.8[b]	2.01 (1.80, 2.22)	122.0 ± 32.0[b]	1.82 (1.72, 1.92)
% of control AUC	100	205		183	
C_{max} ($\mu g\ ml^{-1}$)	16.2 ± 5.2	19.1 ± 4.5[c]	1.20 (1.09, 1.32)	18.3 ± 5.1[d]	1.15 (1.06, 1.24)
% of control C_{max}	100	122		116	
$t_{1/2}$ (h)	2.4 ± 0.5	3.2 ± 0.7[c]		3.1 ± 0.3[d]	
% of control $t_{1/2}$	100	143		134	
T_{max} (h)	1 (0.5–4.0)	2.5 (1.0–4.0)		3 (1.0–4.0)[d]	
R-(−)-Ibuprofen					
AUC ($\mu g \cdot h\ ml^{-1}$)	44.5 ± 8.2	53.0 ± 14.1[d]	1.17 (1.06, 1.30)	51.1 ± 12.4	1.13 (1.03, 1.25)
% of control AUC	100	120		115	
C_{max} ($\mu g\ ml^{-1}$)	15.4 ± 3.9	14.7 ± 3.3	0.96 (0.85, 1.08)	14.9 ± 4.4	0.95 (0.86, 1.06)
% of control C_{max}	100	97		97	
$t_{1/2}$ (h)	1.6 ± 0.2	1.5 ± 0.3[c]		1.7 ± 0,2	
% of control $t_{1/2}$	100	93		106	
T_{max} (h)	1 (0.5–4)	2.25 (1.0–4.0)		1.75 (1.0–4.0)	

[a] The results are means ± standard deviations (median [range] for T_{max}). Vori, pretreatment with voriconazole; and Fluco, pretreatment with fluconazole.
[b] Significantly ($P < 0.001$) different from the results for the control.
[c] Significantly ($P < 0.01$) different from the results for the control.
[d] Significantly ($P < 0.05$) different from the results for the control.

In the control phase, the mean AUCs of *S*-(+)- and *R*-(−)-ibuprofen in the two subjects with the *CYP2C9*1/*3* and *CYP2C8*1/*1* haplotype were 75.8 and 41.1 $\mu g \cdot h\ ml^{-1}$, respectively, which were similar to the mean AUCs of *S*-(+)- and *R*-(−)-ibuprofen in that phase (Table 1). However, these two subjects had the longest elimination half-lives of *S*-(+)-ibuprofen in the control phase, and the prolongation of the elimination $t_{1/2}$ of *S*-(+)-ibuprofen in the voriconazole and the fluconazole phases was the shortest with these subjects (Fig. 2c). The one subject with the *CYP2C9*1/*1* and *CYP2C8*1/*3* haplotype had the highest C_{max} and AUC of *S*-(+)-ibuprofen in the control phase. In addition, he seemed to have the strongest inhibitory effect by both voriconazole and fluconazole. The pharmacokinetic parameters of *S*-(+)- and *R*-(−)-ibuprofen in individuals with different *CYP2C9* and *CYP2C8* haplotypes are illustrated in Fig. 2 and 3.

DISCUSSION

The present study shows that both voriconazole and fluconazole significantly increase the plasma *S*-(+)-ibuprofen concentrations but have only a weak effect on the pharmacokinetics of *R*-(−)-ibuprofen. Voriconazole, at a typical dose used clinically, doubled the exposure to *S*-(+)-ibuprofen (AUC) compared to the level of exposure in the control phase. The greater AUC of *S*-(+)-ibuprofen was apparent in all 12 subjects. Furthermore, the C_{max} and the half-life of *S*-(+)-ibuprofen were higher in the voriconazole phase. Similarly, the mean AUC of *S*-(+)-ibuprofen was 1.8-fold higher after pretreatment with fluconazole, and the half-life and the C_{max} of *S*-(+)-ibuprofen were also higher in the fluconazole phase compared to the values in the control phase. The significant interaction between fluconazole or voriconazole and *S*-(+)-ibuprofen was

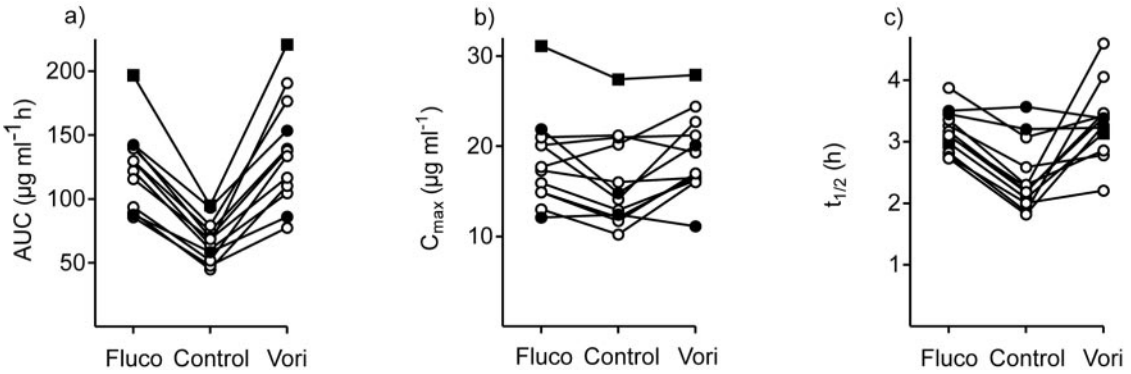

FIG. 2. *S*-(+)-Ibuprofen AUC (a), C_{max} (b), and elimination $t_{1/2}$ (c) values after the administration of 400 mg racemic ibuprofen either alone (Control) or after pretreatment with fluconazole (Fluco) or voriconazole (Vori). Symbols: ●, *CYP2C9*1/*3* and *CYP2C8*1/*1* haplotype; ■, *CYP2C9*1/*1* and *CYP2C8*1/*3* haplotype; ○, *CYP2C9*1/*1* and *CYP2C8*1/*1* haplotype.

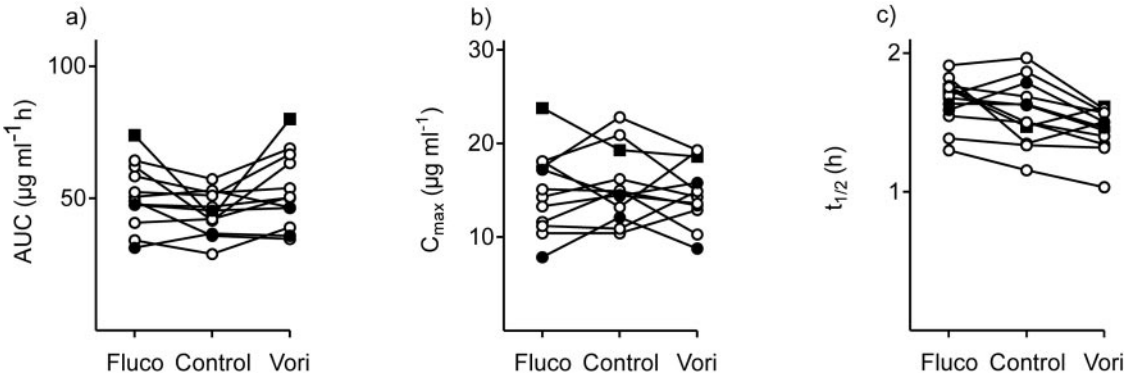

FIG. 3. *R*-(−)-Ibuprofen AUC (a), C_{max} (b), and elimination $t_{1/2}$ (c) values after the administration of 400 mg racemic ibuprofen either alone (Control) or after pretreatment with fluconazole (Fluco) or voriconazole (Vori). Symbols: ●, *CYP2C9*1/*3* and *CYP2C8*1/*1* haplotype; ■, *CYP2C9*1/*1* and *CYP2C8*1/*3* haplotype; ○, *CYP2C9*1/*1* and *CYP2C8*1/*1* haplotype.

also ascertained by bioequivalence testing. This interaction between fluconazole and *S*-(+)-ibuprofen observed in our study is quite similar to the interaction previously documented between fluconazole and another NSAID that is a substrate for CYP2C9, celecoxib, which resulted in 134% increase in the AUC of celecoxib (7).

The most likely mechanism for the interaction between voriconazole or fluconazole and *S*-(+)-ibuprofen is the inhibition of CYP2C9, as this isoenzyme is mainly responsible for the metabolism of *S*-(+)-ibuprofen (12). Voriconazole has been shown to be an inhibitor of the CYP3A4 (31), CYP2C9 (28), and CYP2C19 (http://www.emea.eu.int./humandocs/Humans/EPAR/vfend/vfend.htm) isoenzymes. For example, pretreatment with voriconazole at 400 mg twice daily increased by 80% the mean steady-state AUC of phenytoin, a substrate for CYP2C9 and CYP2C19 (30). Fluconazole is another well-known potent inhibitor of CYP2C9 and CYP2C19 and a moderate inhibitor of CYP3A4 (4, 17, 23). Treatment with fluconazole (400 mg daily) for 6 days inhibited the CYP2C9-dependent hydroxylation of *S*-warfarin by 70% (4). Neither voriconazole nor fluconazole is known to inhibit CYP2C8, the principal enzyme responsible for *R*-(−)-ibuprofen hydroxylation (13, 33). This is supported by the present results, which indicate that these azole antifungals have very little effect on the oral pharmacokinetics of *R*-(−)-ibuprofen. Furthermore, our results suggest that CYP2C9, CYP2C19, and CYP3A4 have little role in the metabolism of *R*-(−)-ibuprofen.

In our study we used the clinically recommended dosages of azole antifungals, which are 200 mg to 400 mg every 12 h and 50 mg to 400 mg every 24 h for voriconazole and fluconazole, respectively. In both phases, a significant correlation between the azole antifungal trough concentrations and the increase in the level of exposure to *S*-(+)-ibuprofen was seen. When it is taken into account that the voriconazole pretreatment consisted of only four doses and that the fluconazole pretreatment consisted of only two doses, it is possible that this kind of short pretreatment did not produce maximal hepatic enzyme inhibition and a longer pretreatment period would have led to an even greater interaction with *S*-(+)-ibuprofen.

Kirchheiner et al. have identified an association between the CYP2C9 polymorphism and decreased *S*-(+)-ibuprofen clearance but not *R*-(−)-ibuprofen clearance (19). Nevertheless, it should be kept in mind that the unidirectional conversion of *R*-(−)-ibuprofen to *S*-(+)-ibuprofen occurs in vivo (2, 16). The apparent oral clearance of *S*-(+)-ibuprofen was approximately halved in carriers of the *CYP2C9*3/*3* genotype, an effect that is similar in magnitude to those found with voriconazole or fluconazole in the present study. Interestingly, it was recently found that the clearance for *S*-(+)-ibuprofen is influenced by *CYP2C8*3* and *CYP2C9*3* alleles to similar extents (11), which suggests the involvement of CYP2C8 on the hydroxylation of *S*-(+)-ibuprofen as well. In our study, 2 of the 12 subjects were carriers of the *CYP2C9*1/*3* and *CYP2C8*1/*1* haplotype. Their elimination $t_{1/2}$ of *S*-(+)-ibuprofen was the longest among the 12 subjects in the control phase. In one of these two subjects, pretreatment with azole antifungals did not produce any prolongation of his *S*-(+)-ibuprofen elimination $t_{1/2}$, and in the other subject, pretreatment with azole antifungals produced only a slight prolongation of his elimination $t_{1/2}$ of *S*-(+)-ibuprofen, which suggests that these two individuals were less prone to the inhibitory effect of CYP2C9 by voriconazole or fluconazole, probably due to lower baseline CYP2C9 activity. The haplotype *CYP2C8*1/*3* and *CYP2C9*1/*1* was found in one of the subjects. He had the highest C_{max} and AUC of *S*-(+)-ibuprofen in the control phase, and he had also the greatest AUC values for both *S*-(+)- and *R*-(−)-ibuprofen in the voriconazole phase and in the fluconazole phase. Accordingly, it seems that the *CYP2C8*1/*3* genotype affects the metabolism of *S*-(+)-ibuprofen, too, as assumed by Garcia-Martin et al. (11).

Coadministration of voriconazole or fluconazole and ibuprofen is likely in a clinical setting. It has been shown that increased *S*-(+)-ibuprofen concentrations due to a lack of functional CYP2C9 enzyme leads to increased inhibition of both cyclooxygenase type 1 and cyclooxygenase type 2, as evidenced by reduced thromboxane B_2 and prostaglandin E_2 formation (19). Accordingly, increased exposure to ibuprofen due to inhibition of CYP2C9 by voriconazole or fluconazole might increase the risk of the concentration-dependent renal, cardiovascular, or gastrointestinal adverse drug reactions caused by ibuprofen. This assumption is supported by the findings of a study reporting that the impaired metabolism of ibuprofen due to mutations in the CYP2C9 gene increases the risk of acute gastrointestinal bleeding (21). On the basis of the results of

earlier studies and those of the present study, the use of a reduced dose of ibuprofen should be considered when it is coadministered with voriconazole or fluconazole.

Visual disturbances have been associated with voriconazole treatment, and the appearance of this adverse effect is dependent on the CYP2C19 genotype, the principal enzyme responsible for the metabolic clearance of voriconazole (14; Mikus et al., Abstr. 7th Congr. Eur. Assoc. Clin. Pharmacol. Ther., 2005). In this open study, one-third of the subjects complained of visual disturbances during voriconazole pretreatment. That proportion is similar to that found in previous studies (29). All the events were mild and resolved within 1 h without any medical intervention. No other drug-related adverse events were recognized during the study.

In conclusion, voriconazole and fluconazole treatments increased the level of exposure to *S*-(+)-ibuprofen 2- and 1.8-fold, respectively. This was likely caused by the inhibition of CYP2C9-mediated biotransformation of *S*-(+)-ibuprofen by voriconazole and fluconazole. The clinical significance of the present fluconazole-ibuprofen and voriconazole-ibuprofen interactions remains unclear. However, patients using high doses of ibuprofen daily are exposed to considerable ibuprofen concentrations if they are treated with voriconazole or fluconazole. Thus, caution should be exercised when treatment with voriconazole, fluconazole, or some other potent CYP2C9 inhibitor is initiated in patients who receive high repeated doses of ibuprofen.

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