Effect of itraconazole on the pharmacokinetics and pharmacodynamics of a single oral dose of brotizolam

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Aims

To assess the effect of itraconazole, a potent inhibitor of cytochrome P450 (CYP)3A4, on the single oral dose pharmacokinetics and pharmacodynamics of brotizolam.

Methods

In this randomized, double-blind, cross-over trial 10 healthy male subjects received either itraconazole 200 mg or matched placebo once daily for 4 days. On day 4, a single 0.5 mg dose of brotizolam was administered orally. Plasma concentrations of brotizolam were followed up to 24 h, together with assessment of psychomotor function measured by the digit symbol substitution test (DSST), visual analogue scales and UKU side-effect rating scale.

Results

Itraconazole significantly ($P < 0.001$) decreased the apparent oral clearance (CL/F) (16.47 ± 4.3 vs 3.91 ± 2.1), increased the area under the concentration-time curves (AUC) from 0 h to 24 h (28.37 ± 10.8 vs 68.71 ± 24.1 ng ml⁻¹), and prolonged the elimination half-life (4.56 ± 1.4 vs 23.27 ± 10.3 h) of brotizolam. The AUC(0,24 h) of the DSST ($P < 0.001$) and the item 'sleepiness' of UKU ($P < 0.05$) were significantly decreased.

Conclusions

Itraconazole increases plasma concentrations of brotizolam probably via its inhibitory effect on CYP3A4 brotizolam metabolism.

Introduction

Brotizolam, one of the triazolothienodiazepine derivatives, is widely used as a short acting hypnotic agent in Japan and Europe. Brotizolam is rapidly and completely absorbed after oral administration, with an elimination half-life in the range of 3–6 h [1]. Bioavailability of brotizolam is assumed to be around 65% based on calculating the ratio of the dose-normalized cumulative amount excreted in four subjects [2]. Brotizolam is metabolized almost completely into hydroxylated compounds identified as α -hydroxybrotizolam and 6-hydroxybrotizolam [3]. α -hydroxybrotizolam is almost

as equi-effective as brotizolam but 6-hydroxybrotizolam has less activity than α -hydroxybrotizolam [4]. All hydroxy metabolites are almost completely conjugated to glucuronic acid and/or to sulfolic acid, which are rapidly excreted in urine [3, 4]. A recent paper reported that the brotizolam was metabolized to α -hydroxybrotizolam and 6-hydroxybrotizolam by CYP3A4 in human liver microsomes [5]. It is well known that triazolam and midazolam, which are benzodiazepines used as hypnotic agents, are metabolized by the CYP3A subfamily *in vitro* [6]. Furthermore, we reported that erythromycin, an inhibitor of CYP3A4, inhibits the metabolism of

alprazolam in healthy volunteers, which provided the first *in vivo* evidence for the involvement of CYP3A4 in the metabolic pathway of alprazolam [7]. The widely used azole antifungal agents ketoconazole and itraconazole are also potent inhibitors of CYP3A4 [8–10]. These antifungal agents strongly interact with orally ingested midazolam and triazolam, increasing the AUC values of these drugs by more than 10 fold [11, 12]. It is considered likely that brotizolam would interact with these antifungal agents but there are no *in vivo* data demonstrating this. In this study, we examined the effect of itraconazole on the pharmacokinetics and pharmacodynamics of brotizolam in healthy volunteers.

Methods

Subjects

The subjects were 10 healthy male volunteers. Their mean $\pm$ SD age was 33.7 ± 5.2 years and body weight 62.3 ± 4.6 kg. Their normal health status was confirmed by medical history, physical examination and haematology and blood chemistry tests. Five subjects were smokers (≥ 10 cigarettes day⁻¹), and the remaining five were non-smokers. None of the subjects used any concomitant medications. The study protocol was approved by the Ethics Committee of Hirosaki University Hospital, and each subject provided written informed consent to participate.

Protocol

The study was performed as a randomized, double-blind cross-over trial, with a washout period of 6 weeks between the two phases. The subjects took either itraconazole (200 mg) as a capsule formulation (Itrazole®, Janssen-Kyowa Co. Ltd, Tokyo, Japan) or matched placebo (as a capsule formulation with the same appearance and size as the itraconazole) once a day (between 11.30 and 12.30 h) for 4 days. On day 4, the subjects received a single oral 0.5 mg dose of brotizolam (Rendormin®, Japan Boehringer Ingelheim Co. Ltd. Tokyo, Japan) 1 h after the last dose of itraconazole or placebo. No food was allowed until 3 h after brotizolam dosing. The blood samples (10 ml each) were collected by venepuncture into heparinized tubes at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after the brotizolam dosing. The blood samples were immediately centrifuged ($1900 \times g$) at ambient temperature and the plasma layer was separated and stored at -40°C until analysis.

Data analysis of pharmacodynamics

Psychomotor function status was evaluated at the same time points as blood samplings in both the control and itraconazole periods. This was done using the digit sym-

bol substitution test (DSST), the number of digits correctly substituted with simple symbols, adapted from the Wechsler Adult Intelligence Scale in 3 min, the items 'thinking speed' of visual analogue scale (VAS) of mood and subjective states employed in the pharmacodynamic assessment study of chlorthalidopoxide [13], and the item 'sleepiness' of the Udvalg for kliniske undersøgelser (UKU) side-effect rating scale [14]. The DSST variables were assessed by changed scores from baseline. The areas under the response-time curves (AUC) were determined by the trapezoidal rule for 0–24 h for each pharmacodynamic variable. Plots of effect-concentration data were made for mean values during each treatment. Effects-concentration data from DSST and UKU were inspected for the potential of pharmacodynamic modelling.

Drug assay

Plasma brotizolam concentrations were measured in duplicate by a high-performance liquid chromatography (HPLC) method developed in our laboratory [15]. Triazolam (5 ng) was added to the plasma samples (1 ml) as an internal standard, then the samples were diluted with 5 ml of 1 M sodium chloride and the solution was briefly mixed. The mixture was applied to a Sep-Pak C₁₈ cartridge. The cartridge was then washed with 10 ml of water and 5 ml of 20% acetonitrile in water. The desired fraction was eluted with 5 ml of 40% acetonitrile in water. The eluent was evaporated to dryness at 60°C . The residue was reconstituted in 100 μl of mobile phase. The samples were injected onto the HPLC. The lowest limit of detection was 0.5 ng ml^{-1} , and the coefficients of variation (both intra-assay and interassay) were less than 8.9%. Plasma itraconazole concentrations were measured using a solid phase extraction combined with the HPLC analysis method developed in our laboratory [16]. Bifonazole (30 ng) was added to the plasma samples (1 ml) as an internal standard, then the samples were diluted with 5 ml of 1 M sodium chloride and the solution briefly mixed. The mixture was applied to a Sep-Pak C₈ cartridge. The cartridge was then washed with 5 ml of water and 5 ml of 40% acetonitrile in water. The desired fraction was eluted with 5 ml of 80% acetonitrile in water. The eluent was evaporated to dryness at 60°C . The residue was reconstituted in 60 μl of ethanol and 40 μl of mobile phase. The samples were injected onto the HPLC. The HPLC column used was a Grand Pack C₈-5 (MAIS Inc. Owani, Japan) with mobile phase consisting of 0.5% KH₂PO₄ (pH 4.5) : acetonitrile = 47 : 53. An ultraviolet detector was used to detect itraconazole and bifonazole. The

detection wavelength was set at 263 nm. The recovery of the extraction method was over 89.1% at several itraconazole concentrations. The lowest limit of detection was 5 ng ml⁻¹ and the coefficients of variation (both intra-assay and interassay) were less than 6.9%.

Data analysis of pharmacokinetics

The elimination rate constant (λ_z) of brotizolam was estimated using linear regression analysis of the terminal log-linear concentration-time data, and the elimination half-life ($t_{1/2}$) was calculated from $0.693/\lambda_z$. The area under the plasma concentration-time curve from 0 h to infinity (AUC(0,∞)) or total AUC, was calculated from AUC(0,24 h) + C_{24}/λ_z , where C_{24} is the plasma concentration of brotizolam at 24 h after the dosing. The apparent oral clearance (CL/F) was calculated from dose/total AUC. The peak plasma concentration (C_{max}) and the time to C_{max} (t_{max}) were determined graphically.

Statistical analysis

Results are expressed as mean values ± SD and the 95% confidence intervals for major differences between the phases. Pharmacokinetic and pharmacodynamic variables between the groups were compared by one-way ANOVA. ANOVA followed by Tukey's test was used for pharmacokinetic analysis based on log-transformed data, except for t_{max} , and ANOVA was also used for pharmacodynamic values. A significant difference was accepted when $P < 0.05$.

Results

The mean plasma brotizolam concentration-time data during treatment with placebo or itraconazole are shown in Figure 1. The mean (95% confidence interval) pharmacokinetics parameters are summarized in Table 1. When brotizolam was administered 1 h after the last

dose of itraconazole, the oral clearance (CL/F) of brotizolam was reduced 0.23 fold (95% CI 0.14, 0.32; $P < 0.001$), the AUC(0,24 h) was increased 2.6 fold (95% CI 1.87, 3.39; $P < 0.002$) and the elimination $t_{1/2}$ increased 5.1 fold (95% CI 3.7, 6.5; $P < 0.001$) compared with control values. The t_{max} of brotizolam was also increased 4.2 fold (95% CI 2.4, 6.0; $P < 0.01$) during itraconazole treatment, but the C_{max} was not significantly higher. The second peak of brotizolam during itraconazole treatment probably originated from interindividual differences of measurement values. Plasma itraconazole concentration-time data on day 4 are shown in Figure 2. The mean maximum concentration of itraconazole was 324.2 ng ml⁻¹. There was a 5-fold interindividual variation in the C_{max} of itraconazole. The mean

Figure 1

Plasma concentrations of brotizolam (mean ± SD) after an oral dose of 0.5 mg brotizolam following daily pretreatment with itraconazole 200 mg (●) or placebo (○) for 4 days in 10 healthy volunteers

Table 1

The pharmacokinetic variables of 0.5mg oral brotizolam (95% confidence interval, (95% CI)) in 10 subjects after pretreatment with placebo or 200mg itraconazole once daily for 4 days

Variable	Placebo (95% CI)	Itraconazole (95% CI)	Significance
C_{max} (ng ml ⁻¹)	4.82 (3.86, 5.75)	5.94 (4.42, 7.45)	NS
t_{max} (h)	0.75 (0.44, 1.05)	3.20 (1.86, 4.53)	$P < 0.05$
Elimination $t_{1/2}$ (h)	4.51 (3.67, 5.34)	23.27 (16.86, 29.65)	$P < 0.001$
CL/F (l h ⁻¹)	16.40 (13.70, 19.21)	3.91 (2.50, 5.31)	$P < 0.001$
AUC(0,24 h) (ng ml ⁻¹ h)	28.37 (21.64, 35.09)	68.71 (53.77, 83.64)	$P < 0.002$
AUC(0,∞) (ng ml ⁻¹ h)	33.10 (25.71, 40.40)	168.67 (110.20, 226.92)	$P < 0.001$

Figure 2

Plasma concentrations (mean $\pm$ SD) of itraconazole in 10 healthy volunteers on day 4

itraconazole concentration was over the 200 ng ml⁻¹ at time 0 and over 100 ng l⁻¹ after 24 h in the co-administration study. The mean AUCs (0,24 h) for each psychomotor function parameter are shown in Figure 3. The CNS-depressant effects of brotizolam, assessed by several psychomotor function tests, were observed for 24 h with recovery evident 24 h after drug administration. There were significant differences in the pharmacodynamic parameters (AUC(0,24 h)) of DSST (-3.8; 95% CI -7.41, 0.65; vs 9.1; 95% CI 5.06, 13.13, $P < 0.001$) and the item 'sleepiness' of UKU (0.94; 95% CI 0.74, 1.14; vs 1.34; 95% CI 1.13, 1.54; $P < 0.05$) between the control and itraconazole arms of the study (Figure 3). No differences were found in AUC for any pharmacodynamic variables in VAS including the item of 'thinking' (-12.4; 95% CI -15.58, -9.36; vs -16.99; 95% CI -20.10, -13.89; $P = 0.05$) and 'Spacy' (-4.61; 95% CI -7.41, -1.81; vs -1.61; 95% CI -4.41, 1.19; $P = 0.123$) (Figure 3). A plot of mean change of DSST and UKU vs mean brotizolam plasma concentration shown in

Figure 3

Results of the median score change in (a) digit symbol substitution test (DSST), (b) UKU side-effect rating scale (the item sleepiness), (c) VAS (thinking speed) and (d) VAS (Spacy) after an oral 0.5 mg dose of brotizolam following pretreatment with itraconazole (●) or placebo (○)

Figure 4

Plots of mean change in DSST in (a) control, (b) itraconazole and raw UKU score in (c) control, (d) itraconazole vs mean brotizolam plasma concentration

Figure 4 illustrates counter-clockwise hysteresis in both cases.

Discussion

Recent studies have uncovered many potentially hazardous interactions associated with the use of itraconazole, e.g. interactions with oral midazolam [11], triazolam [12] and zopiclone [17]. In the present study, itraconazole decreased CL/F , increased the $AUC(0,24\text{ h})$, and prolonged the elimination $t_{1/2}$ of brotizolam (Table 1). The results of the psychomotor tests were in good agreement with the pharmacokinetics findings (Figure 3). These findings provide further *in vivo* evidence of the involvement of CYP3A4 in brotizolam metabolism [5].

Previous studies have shown that itraconazole, in a dose of 200 mg day⁻¹, increased the mean $AUC(0,\infty)$ of triazolam by 27-fold [12], with significant linear correlation between the plasma concentration of itraconazole and AUC of triazolam [18]. Similarly, midazolam concentrations have increased 10-fold [11], supported by *in vitro* evidence of high inhibitory activity of itraconazole for CYP3A4 catalyzed hydroxylation of midazolam [19]. The concentrations of itraconazole in the present study were maintained at over 100 ng ml⁻¹ until after

24 h, which appears to be high enough to inhibit the metabolism of brotizolam catalyzed by CYP3A4 *in vivo*.

Previous clinical studies suggested that itraconazole inhibited P-glycoprotein-mediated drug transportation [20, 21]. A recent *in vitro* study supported these results [22]. However, since two other benzodiazepines, midazolam and flunitrazepam, were not transported by P-glycoprotein *in vitro* [23, 24], it is unlikely that brotizolam is a clinically significant P-glycoprotein substrate.

Itraconazole altered not only the pharmacokinetics of brotizolam but also psychomotor function affected by brotizolam (Figure 3). The more depressed psychomotor function during itraconazole co-administration is probably explained by the elevated plasma concentrations of brotizolam. Physiological explanation for counter-clockwise hysteresis in pharmacodynamics vs plasma concentration plots (Figure 4) include delayed distribution to the effect site, delayed effect and sensitization or fatigue. Chronic tolerance also appeared to develop with itraconazole treatment. Previous papers have reported the development of tolerance to the pharmacodynamic effects during continuous and con-

stant plasma concentration of benzodiazepines [25, 26]. Therefore, the tolerance of brotizolam effects may be due to continuously high plasma concentrations of brotizolam by metabolic inhibition with itraconazole.

In conclusion, itraconazole significantly increases the plasma concentrations of brotizolam, probably through inhibition of CYP3A4, the major metabolic enzyme responsible for *in vivo* brotizolam elimination. Careful choice of dose of brotizolam is necessary in patients receiving itraconazole.

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