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SHORT COMMUNICATION

Pharmacokinetics and Electroencephalographic Effects of Ketoconazole in the Rat

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ABSTRACT: To evaluate methodology for *in vivo* interaction studies of benzodiazepines (BZs) and ketoconazole (KCZ) in animal models, this study assessed the pharmacokinetics and electroencephalographic (EEG) effect of KCZ, and suitable dosage regimens of KCZ to maintain sufficiently high KCZ concentrations to inhibit metabolism of BZs in rats. Rats were injected intraperitoneally (i.p.) with KCZ 10 mg kg^{-1}. No significant EEG change was detected regardless of serum KCZ concentration, indicating that the EEG changes after both BZ and KCZ administration can be attributed entirely to BZ. Serum KCZ concentrations showed an apparent nonlinear pattern of decline with a short half-life (1.38 h). An additional dose of 5 mg kg^{-1} i.p. given 180 min after the initial dose sustained KCZ concentrations above 2 μg mL^{-1} until at least 500 min after the initial dose. These results provide the basis for design of animal models for *in vivo* assessment of interactions of BZs and KCZ. Copyright © 1999 John Wiley & Sons, Ltd.

Key words: ketoconazole; pharmacokinetics; electroencephalogram

Introduction

Quantitative analysis of electroencephalogram (EEG) has proven to be a useful approach to evaluate the concentration–effect relationship for benzodiazepines (BZs) in animals [1] and humans [2–5]. Among factors that may affect these relationships are drug interactions. Azole antifungal agents (ketoconazole, itraconazole, etc.) are known to inhibit metabolism of BZs. Previous studies reported that ketoconazole (KCZ), a potent cytochrome P-450 (CYP) 3A inhibitor, increased plasma triazolam concentrations and enhanced the EEG effect of triazolam in humans [6,7]. However, it is still not completely established whether KCZ itself produces BZ agonist effects. In two clinical studies [6,7], typical therapeutic doses of KCZ produced no detectable EEG changes in human volunteers. Nonetheless, KCZ is a BZ receptor ligand [8], and in behavioral studies some degree of agonist activity could not be excluded [8].

Although the mechanism of CYP 3A inhibition by KCZ is not clearly identified, a number of studies indicate that KCZ inhibits CYP 3A by a mechanism that is fully or partly competitive [6,9,10]. After a single oral dose of KCZ in rats, plasma and liver KCZ concentrations decline with time approximately in parallel [11], suggesting that hepatic CYP 3A inhibition by KCZ may likewise decline with plasma KCZ levels after single oral or parenteral doses. For *in vivo* studies assessing inhibition of metabolism of BZs or other compounds by KCZ, the KCZ dosage regimen should produce plasma and liver KCZ concentrations that are sufficient to cause CYP 3A inhibition.

To address these issues, this study assessed (1) the electroencephalographic effect of KCZ in relation to its pharmacokinetics, and (2) dosage regimens of KCZ needed to maintain sufficiently high KCZ concentrations to inhibit metabolism of BZs.

Materials and Methods

EEG electrodes were implanted onto the skull in the rat ($n = 4$, Male Sprague-Dawley, 300–350 g) several days before the experiment. An indwelling cannula for blood sampling was also implanted in the right jugular vein. The surgery was performed under ketamine/xylazine anesthesia. Bipolar EEG leads were continuously measured during the experiment using an EEG instrument (Grass Instrument Co., MA, USA). The 60-s EEG segments were recorded before and every 15 min until 360 min after intraperitoneal (i.p.) administration of KCZ 10 mg kg^{-1} (dissolved in polyethylene glycol 400,

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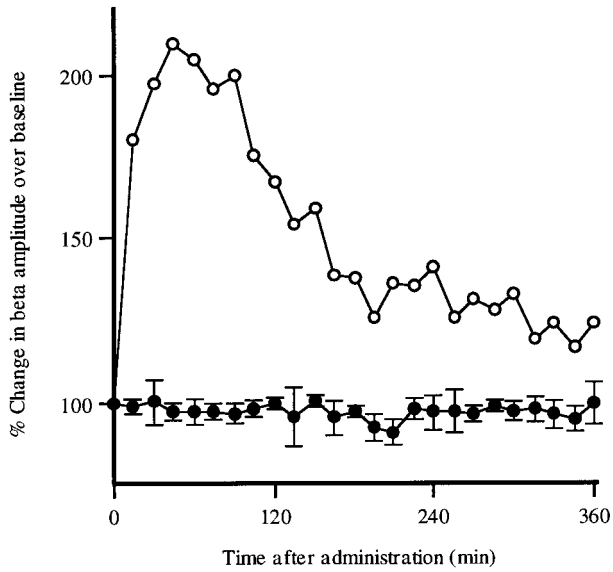

Figure 1. Percentage change over baseline value of the amplitude in 11.5–30 Hz (left hemisphere) after i.p. administration of ketoconazole 10 mg kg^{-1} in rats (mean ± S.D., $n = 4$). Open circles shows typical EEG changes by midazolam (2 mg kg^{-1}, i.v. dose) in another study

10 mg mL^{-1}). Rats were kept awake during EEG recording sessions by oscillating their cages using a shaker (New Brunswick Scientific Co. Inc., NJ, USA). EEG signals were subjected to fast Fourier analysis using RHYTHM 9.0 (Stellate Systems, Quebec, Canada), and the average amplitudes (μV) of 1.5–3.5 Hz (δ), 4–7.5 Hz (θ), 8–12.5 Hz (α) and 13–31 Hz (β) were calculated. Percentage changes in these average amplitudes over baseline value were used to describe the drug effects.

Blood samples (100–400 μL) for KCZ measurement were collected before and 15, 45, 75, 120, 180, 240, 300 and 360 min after drug administration. Serum KCZ concentrations were measured by high performance liquid chromatography using a UV detector [6]. The rat serum was free from substances that interfered with the peaks of interest. The lower limit of sensitivity was 0.25 μg mL^{-1} for a 200 μL serum sample. The slope (K_e) of the terminal log-linear phase of the serum concentration curve was used to calculate the elimination half-life ($t_{1/2}$). The area under the serum concentration curve from zero up to the final detectable concentration was measured by use of the linear trapezoidal method and extrapolated to infinity ($AUC_{0-\infty}$). Apparent oral clearance (Cl/F) was calculated as dose divided by AUC. Apparent volume of distribution (V_d/F) using the area method was calculated as Cl/F divided by K_e. Based on the single dose pharmacokinetics of KCZ, two dosing regimens of KCZ were evaluated; a single 10 mg kg^{-1} i.p. dose, or the same initial dose followed by a 5 mg kg^{-1} i.p. dose 180 min later.

Results and Discussion

Average amplitude in the beta band did not increase significantly over predose baseline (Figure 1) regardless of serum KCZ concentrations (Figure 2). None of other three frequency bands showed significant changes (data are not shown). These results indicated that the EEG changes after both BZ and KCZ administration can be attributed entirely to BZ at least within the range of KCZ concentration shown in this study. This is consistent with observations in humans [6,7], and with experimental data

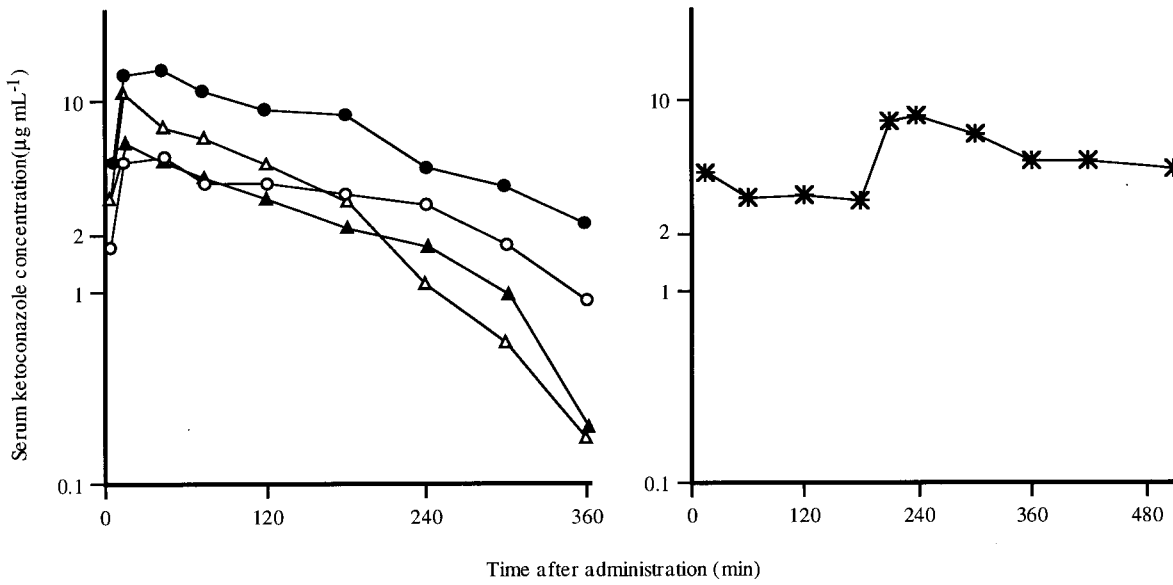

Figure 2. Left panel, serum concentration–time curve of ketoconazole after single i.p. doses of ketoconazole 10 mg kg^{-1} in individual rats; right panel, a serum concentration–time curve of ketoconazole after a single i.p. administration of 10 mg kg^{-1} followed by 5 mg kg^{-1} given 180 min after the initial dose in a rat

Table 1. Pharmacokinetic parameters of ketoconazole after i.p. dose of ketoconazole 10 mg kg^{-1} in rats ($n = 4$)

Parameter	Mean ± S.D.
Peak serum concentration (μg mL^{-1})	9.2 ± 4.5
Time of peak (h)	0.50 ± 0.29
$AUC_{0-\infty}$ (μg · h mL^{-1})	26.8 ± 15.7
Cl/F (L h^{-1} kg^{-1})	0.45 ± 0.18
V_d/F (L kg^{-1})	0.89 ± 0.57
$t_{1/2}$ (h)	1.38 ± 0.52

indicating that KCZ is a BZ receptor ligand having neither agonist or inverse agonist properties [8]. It is possible that a delay in KCZ distribution to the brain could contribute to the undetectable EEG effect. However, brain KCZ concentrations were approximately one-third of serum concentrations, and declined with time almost in parallel to serum concentrations after intragastric administration of KCZ in mice [12]. The vehicle used here, polyethylene glycol 400, causes no EEG change even after i.v. administration (unpublished data).

The serum KCZ concentration–time curve reached a maximum at approximately 0.5 h after dosage (Figure 2) with subsequent elimination having a short half-life (Table 1). Matthew *et al*. reported the pharmacokinetics of i.v. KCZ in rats, in which nonlinear kinetics in AUC, Cl and V_d were observed as the dose was escalated [13]. In humans, a similar disproportionate increase in AUC has been noted following oral doses of 100–800 mg [14]. This nonlinearity has been interpreted as indicating saturable hepatic metabolism. The metabolic pathway of KCZ includes oxidation of imidazole ring, degradation of the oxidized imidazole, oxidative *O*-dealkylation, oxidative degradation of the piperazine ring and aromatic hydroxylation [14]. In mice, *N*-deacetyl KCZ appears to be the major metabolite [15], and in rat hepatic microsomes, this metabolite is further metabolized by flavin-containing monooxygenase [16]. However, metabolic pathways in humans are not completely understood. The authors observed an apparent nonlinear pattern of decline in serum KCZ concentrations at lower levels (Figure 2), which is in agreement with previous observations after single i.v. doses of KCZ 5 or 10 mg kg^{-1} [13]. Matthew *et al*. calculated Michaelis–Menten parameters of KCZ pharmacokinetics using constant rate infusions [13]. They obtained a K_m value (the drug concentration producing 50% of the maximum velocity of metabolism) of 2.1 μg mL^{-1}. This corresponds to the concentration as early as 180 min after administration in this study (Figure 2). The pharmacokinetic behavior of KCZ, therefore, might complicate the interpretation of drug interaction data. Figure 2 also shows a representative KCZ concentration–time profile after an i.p. dose of KCZ 10 mg kg^{-1}, followed by 5 mg kg^{-1} given 180 min after the initial dose. KCZ concentrations of at least 2 μg mL^{-1} were maintained until at least 500 min after administration. In the case of single doses, this level was maintained only until 180 min after administration. In humans, KCZ concentrations exceeding 2 μg mL^{-1} are sufficient to produce 90% or greater impairment in clearance of orally administered triazolam [6,7] or midazolam [9]. Assuming BZ clearance is similarly impaired by KCZ in rats, this experimental dosage should be suitable for kinetic and dynamic studies of the interaction of KCZ and BZ.

The i.p. route of administration could affect the bioavailability of KCZ. Remmel *et al*. studied the pharmacokinetics of a single i.v. dose of KCZ 5 mg kg^{-1} [17]. The mean AUC value, calculated from reported Cl value and dose of KCZ, was 5.8 μg · h mL^{-1}. The mean $AUC_{0-\infty}$ of 26.5 μg · h mL^{-1} following a 10 mg kg^{-1} i.p. dose is proportionally larger than this. On the other hand, Matthew *et al*. examined the pharmacokinetics of KCZ after a single i.v. dose of 10 mg kg^{-1}, reporting a truncated AUC(0–120 min) of 2020 mg · min L^{-1} (33.7 μg · h mL^{-1}) [13]. Although this AUC value has not been extrapolated to infinity, it exceeds the AUC value after i.p. dosage. The bioavailability of a single i.p. dose of KCZ 10 mg kg^{-1} cannot be definitely estimated using these data from different laboratories, but probably exceeds 50%.

In recent years, KCZ has been used extensively as a CYP 3A inhibitor in *in vitro* interaction studies. Further *in vivo* interaction studies in animal models and humans would facilitate understanding of the interaction. The results of the current study provide information directly relevant to the design of *in vivo* interaction studies of KCZ and BZ in animal models.

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