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Pharmacokinetics and Dose Proportionality of Ketoconazole in Normal Volunteers

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Ketoconazole is an orally effective, broad-spectrum, systemic antifungal agent. The pharmacokinetics and bioavailability of ketoconazole given as a 200-mg single dose in a tablet, suspension, or solution were studied in 24 fasting healthy males by using a crossover design. Levels of ketoconazole in plasma were determined for up to 48 h by a sensitive reverse-phase high-performance liquid chromatography method. The absorption of ketoconazole was rapid, with mean maximum concentrations of the drug in plasma of 4.2, 5.0, and 6.2 μg/ml attained at 1.7, 1.2, and 1.0 h, respectively, after administration of the tablet, suspension, and solution, respectively. The mean distribution and elimination half-life values were 1.5 to 1.7 and 7.5 to 7.9 h, respectively. The mean oral clearance of the solution dose was 209 (±82.9 [standard deviation]) ml/min, and the mean apparent volume of distribution was 88.31 (±68.72) liters. The relative bioavailabilities for the tablet and suspension were 81.2 (±33.5) and 89.0 (±23.1)%, respectively, of that of the solution. The data indicated the bioequivalence of the tablet to the suspension and of the suspension to the solution. Dose proportionality of ketoconazole was also studied in 12 volunteers after they received solution doses of 200, 400, and 800 mg. Linear correlations between the dose and the maximum concentration of the drug in plasma, the time to the maximum concentration, and the area under the concentration-time curve were observed. However, the increase in the area under the curve was more than proportional to the dose given. The levels in plasma seemed to decay at a lower rate after 400- and 800-mg doses. The mean oral clearance decreased from 244.9 to 123.6 and 80.0 ml/min, respectively, as the dose increased from 200 to 400 and 800 mg. The apparent dose-dependent kinetics may have been due to the presystemic elimination and capacity-limited hepatic metabolism which become saturated at higher doses.

Ketoconazole is a synthetic imidazole-dioxolane derivative with a broad spectrum of antifungal activity. Although its spectrum of activity is similar to those of other imidazole derivatives, e.g., miconazole, econazole, and clotrimazole, ketoconazole has the advantage of being effective when administered orally. Its therapeutic efficacy was reviewed recently (7). After a 200-mg tablet dose to healthy subjects, peak ketoconazole concentrations in plasma (C_{max}) of 3 to 4.5 μg/ml were reached within 1 to 2 h and the therapeutic activity was maintained for several hours after administration of the drug (8). The recommended dosage for ketoconazole is 200 mg daily. In serious infections, higher doses may be required. Ketoconazole is available as a 200-mg tablet (10).

The objectives of this study were (i) to determine the bioavailabilities of the ketoconazole tablet and an experimental suspension formulation relative to a reference aqueous solution and (ii) to evaluate in healthy, male volunteers the dose proportionality of ketoconazole in doses ranging from 200 to 800 mg.

MATERIALS AMD METHODS

Subjects. Our subjects were 24 healthy, nonsmoking, male college students ranging in age from 18 to 25 years (mean, 20 years), in body weight from 61.2 to 95.3 kg (mean, 76.4 kg), and in height from 167.6 to 188 cm (mean, 180 cm). The subjects were judged to be in good health based on thorough prestudy physical examinations and the results from complete hematology, urinalysis, and biochemical tests. The purposes and protocol of the study were explained to them, and they all gave informed consent. On the evening before the study day, the subjects reported to the hospital. Vital signs were monitored at the time of check-in, and the subjects were provided with a standard dinner. The subjects fasted overnight. The drug was administered around 8:00 a.m. on the next day, and a standard lunch was provided about 3 to 4 h later. The subjects were allowed to drink water freely except for a period from 2 h preadministration to 1 h postadministration.

The study protocol was approved by the Review Board for the Protection of Human Subjects in Research at Rutgers-The State University of New Jersey. The investigation was performed at the Hurtado Rutgers Student Health Center, New Brunswick, N.J.

Study design and protocol. The investigation was done in two phases. Phase 1 was a comparative bioavailability study (200-mg single dose) involving 24 subjects, and phase 2 was a dose proportionality study (200-, 400-, and 800-mg solution doses) involving the 12 subjects who had completed phase 1.

Phase 1: relative bioavailability study. The 24 subjects were randomly assigned to two groups. Subject numbers were sequentially assigned at the time of initial drug administration. Ketoconazole (200 mg) in the form of a tablet, suspension (20 mg/ml), or aqueous solution (20 mg/ml) was administered with 200 ml of water to each subject according to a randomized crossover schedule. A washout period of 1 week separated each treatment. Blood samples (10 ml) were collected from an antecubital vein into heparinized

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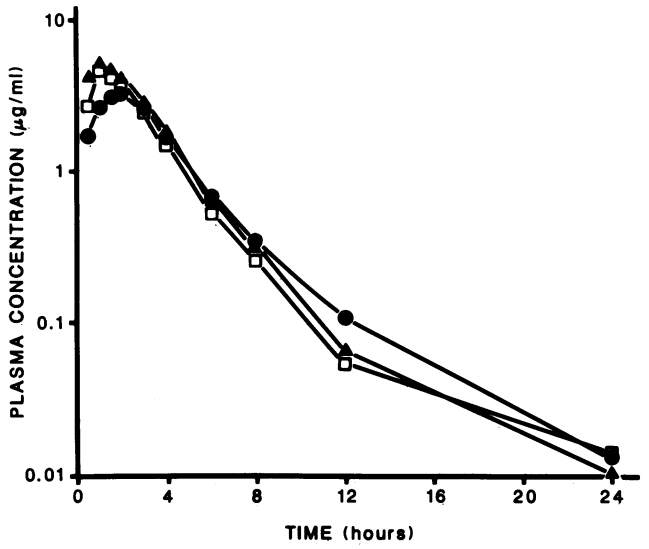

FIG. 1. Mean ketoconazole C_p-t profiles for 23 volunteers after the administration of 200-mg doses. ●, Tablet; □, suspension; ▲, solution.

VACUTAINER tubes (Becton Dickinson Vacutainer Systems, Rutherford, N.J.) through a butterfly catheter or by venipuncture before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h after the administration of ketoconazole. The plasma was separated immediately by centrifugation and stored frozen until analyzed.

Phase 2: dose proportionality study. Twelve subjects who had completed phase 1 were given two additional ketoconazole solution doses (400 mg/20 ml and 800 mg/40 ml) in a randomized crossover sequence. The blood sampling schedule was identical to that of phase 1. The subjects were monitored for any possible adverse effects related to ketoconazole administration.

Drug assay. The plasma samples were analyzed for ketoconazole by a sensitive high-performance liquid chromatographic method. The method involved an extraction procedure with a heptane-isoamyl alcohol (95/5) mixture. The separations were achieved by using a reverse-phase column and a mobile phase of water-acetonitrile-diethylamine (40/60/0.05). Both ketoconazole and terconazole (internal standard) were detected at a wavelength of 254 nm (13). When 2 ml of the plasma sample was extracted and reconstituted in 75 μl of the elution solution and a 40-μl aliquot was injected, the lower limit of detection was 2 ng/ml (with a 30% coefficient of variation). At a plasma concentration (C_p) of 10 ng/ml, which corresponds to the mean C_p observed at 24 h after a 200-mg dose, the precision of the assay was greatly improved (with a 9.8% coefficient of variation).

Pharmacokinetic and statistical analyses. The individual C_p versus time (t) data were first plotted on semilogarithmic papers. The individual elimination rate constant (k_{el}) was calculated by linear regression analysis by using the datum points on the terminal log linear portion of the C_p-t plot. Half-life ($t_{1/2}$) values were calculated by the following equation: $t_{1/2} = 0.693/k_{el}$. The area under the C_p-t curve from time zero to infinity (AUC) was calculated by the linear trapezoidal method up to t (AUC_t) and by extrapolation to infinity, i.e., $AUC = AUC_t + C_p^t/k_{el}$, in which C_p^t is the C_p observed at t, the last sampling time with a measurable C_p.

The relative bioavailability for the tablet or suspension at the 200-mg level in each subject was calculated from the ratio of the AUC value after administration of the tablet or suspension to the AUC value of the solution dose.

The apparent oral clearance (CL/F) and the apparent volume of distribution (V/F) were calculated from the data obtained after administration of the 200-mg solution dose by using the following equations: CL/F = dose /AUC and V/F = (CL/F)/k_{el}, in which F is the fraction of the dose absorbed and entered into systemic circulation.

Statistical analyses of variance were performed on the following parameters obtained after each treatment: (i) C_p at each sampling time, (ii) C_{max}, (iii) the time of peak concentration in plasma (T_{max}), and (iv) the AUC.

To evaluate the dose proportionality of ketoconazole in doses ranging from 200 to 800 mg, linear regression analyses of the pharmacokinetic parameters (C_{max}, T_{max}, AUC, and CL/F) and the dose were performed.

All of the data are expressed as the mean ± the standard deviation.

RESULTS

Phase 1: relative bioavailability study. Twenty-three subjects completed all three treatments required for phase 1. One subject withdrew from the study after one treatment for a reason not related to the drug tested; his data were therefore not included in the analysis. The absorption of ketoconazole after oral administration was rapid. Ketoconazole was detectable in the plasma at 0.5 h after the administration of the tested formulations. The mean C_{max}s were reached as follows: 4.22 μg/ml (200-mg tablet at 1.74 h), 5.04 μg/ml (200-mg suspension at 1.17 h), and 6.17 μg/ml (200-mg solution at 1.02 h). The mean ketoconazole C_p-t profiles are shown in Fig. 1 and Table 1. Of the 69 C_p-t plots, 33 showed multiexponential characteristics after a 200-mg dose. After the C_{max} was reached, the plasma decline was biphasic, with a mean $t_{1/2}$ of 1.7 (±0.6), 1.5 (±0.4), and 1.6 (±0.5) h during the first 8 to 12 h and a mean $t_{1/2}$ of 7.9

TABLE 1. Mean C_p for 23 volunteers after administration of 200-mg doses of ketoconazole

Ketoconazole formulation	C_p (μg/ml) (±SD) after h:									
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12.0	24.0
Tablet	1.71 (±2.55)[a]	2.61 (±2.12)[b]	3.10 (±2.06)[b]	3.26 (±1.96)	2.70 (±1.70)	1.72 (±1.10)	0.67 (±0.51)	0.34 (±0.34)	0.11 (±0.20)	0.01 (±0.01)
Suspension	2.69 (±2.19)	4.66 (±1.61)	4.19 (±1.34)	3.91 (±1.60)	2.46 (±1.10)	1.53 (±0.89)	0.53 (±0.41)	0.25 (±0.26)	0.05 (±0.08)	0.01 (±0.01)
Solution	4.27 (±2.96)	5.16 (±1.88)	4.73 (±1.36)	3.88 (±1.46)	2.67 (±0.98)	1.70 (±0.89)	0.66 (±0.47)	0.34 (±0.29)	0.07 (±0.09)	0.01 (±0.005)

[a] Statistically significantly different ($P < 0.05$) from the solution only, based on analysis of variance and Tukey's multiple range test.
[b] Statistically significantly different ($P < 0.05$) from the suspension and the solution, based on analysis of variance and Tukey's multiple range test.

TABLE 2. Summary of pharmacokinetic and bioavailability data after administration of 200-mg doses of ketoconazole to 23 volunteers[a]

Ketoconazole formulation	C_{max} (μg/ml)	T_{max} (h)	AUC (μg · h/ml)	Relative bioavailability[b]	$t_{1/2}$ (h)
Tablet	4.22 (±2.47)[c]	1.7 (±0.9)[d]	14.74 (±8.48)[c]	0.81 (±0.34)[c]	7.9 (±3.8)
Suspension	5.04 (±1.58)	1.2 (±0.5)	15.84 (±7.05)	0.89 (±0.23)	7.9 (±4.3)
Solution	6.17 (±2.29)	1.0 (±0.4)	18.16 (±6.66)		7.5 (±2.3)

[a] All values are given as the mean ± the standard deviation.
[b] Bioavailability relative to the solution, based on the AUC ratio.
[c] Statistically significantly different ($P < 0.05$) from the solution only, based on analysis of variance and Tukey's multiple range test.
[d] Statistically significantly different ($P < 0.05$) from the suspension and the solution, based on analysis of variance and Tukey's multiple range test.

(±3.8), 7.9 (±4.3), and 7.5 (±2.3) h thereafter after the administration of the tablet, suspension, and solution, respectively (Fig. 1).

Statistical analysis showed that, although there were differences in the early hours (0.5 to 1.5 h) after treatment, there were no statistically significant differences ($P > 0.05$) in the ketoconazole C_ps observed from 2 to 24 h. The suspension and solution formulations exhibited higher rates of absorption than that of the tablet, and the mean ketoconazole C_{max} attained after administration of the solution was significantly higher than that attained after administration of the tablet ($P < 0.05$) (Table 2). However, there was no statistically significant difference in the C_{max} between the suspension and solution formulations. The mean AUCs after each treatment are also shown in Table 2. The mean relative bioavailabilities for the tablet and suspension were 81.0 (±34.0)% and 89.0 (±23.0)% of that of the solution dose, respectively; the difference between the tablet and solution formulations was statistically significant ($P < 0.05$), whereas the difference between the suspension and solution formulations was not.

The mean CL/F, calculated by using the AUC from the 200-mg solution dose, was 209.9 (±82.9) ml/min, and the mean V/F was 88.31 (±68.72) liters, suggesting an extensive distribution of the drug in the body. Since no intravenous injection was made, the absolute bioavailability (F) cannot be evaluated.

Phase 2: dose proportionality study. All 12 subjects completed phase 2. As in the case of the 200-mg dose, ketoconazole was rapidly absorbed after 400- and 800-mg doses. However, the T_{max}s were slightly longer after the 400- and 800-mg doses. The mean C_{max}s of 5.38 (±1.67), 11.77 (±2.35), and 21.83 (±2.84) μg/ml were reached at 0.92 (±0.29), 1.08 (±0.15), and 2.04 (±0.66) h after oral administration of single solution doses of 200, 400, and 800 mg, respectively (Table 3). Except for the C_p at 0.5 h after the 400- and 800-mg doses, the C_ps of ketoconazole observed at all sampling times were significantly different from each other after the 200-, 400-, and 800-mg doses. The ketoconazole C_p seemed to decay at lower rates after the 400- and 800-mg doses than after the 200-mg dose (Fig. 2). For example, the mean ketoconazole C_p at 12 h after the 200-mg dose was 0.044 μg/ml as compared with 0.51 and 4.78 μg/ml at the same time after the 400- and 800-mg doses, respectively. Although only 8 (of 12) subjects had detectable ketoconazole C_ps at 24 h after the 200-mg dose (mean, 0.01 μg/ml), all 12 subjects had detectable ketoconazole C_p at 24 h after the 400- and 800-mg doses (means, 0.03 and 0.21 μg/ml, respectively).

The correlations of C_{max}, T_{max}, and AUC with the doses are shown in Fig. 3. There was a linear increase in the values of these three pharmacokinetic parameters over the dose range studied. Although the AUC increased linearly with the dose, it should be noted that the increase in the AUC was more than proportional. For example, there was a 3.9-fold (2.7- to 4.9-fold) increase in the AUC for a 2-fold increase in dose (from 200 to 400 mg), and a 4-fold increase in dose (from 200 to 800 mg) resulted in a 11.8-fold (6.8- to 15.5-fold) increase in the AUC.

The mean CL/F decreased as the dose increased. It decreased from 244.9 (±96.0) to 123.6 (±31.4) to 80.8 (±17.8) ml/min as the dose increased from 200 to 400 to 800 mg, respectively; the differences in the CL/F between the 200- and 800-mg doses and between the 200- and 400-mg doses were statistically significant (Tukey's test).

DISCUSSION

Single-dose pharmacokinetics and bioavailability of ketoconazole in healthy males after overnight fasting were studied by using a sensitive reverse-phase high-performance liquid chromatography method. Our study compared the bioavailability of a ketoconazole tablet and an experimental suspension formulation to a reference aqueous solution in a relatively large sample population (24 healthy volunteers) for a longer duration (up to 48 h) after each treatment. The results obtained indicated that ketoconazole from both the tablet and suspension formulations was rapidly absorbed on

TABLE 3. Mean C_ps for 12 volunteers after administration of 200-, 400-, and 800-mg solution doses of ketoconazole

Ketoconazole dose (mg)	C_p (μg/ml) (±SD) after h:											
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12.0	24.0	36.0	48.0
200	4.02 (±2.13)	5.04 (±1.37)	4.15 (±1.16)	3.52 (±1.72)	2.37 (±1.15)	1.48 (±1.04)	0.55 (±0.51)	0.27 (±0.26)	0.04 (±0.04)	0.01 (±0.04)[a]	ND[b]	ND
400	7.53 (±2.65)	11.46 (±2.88)	10.46 (±1.87)	9.60 (±1.41)	7.97 (±1.30)	6.75 (±1.57)	3.33 (±1.22)	2.00 (±1.16)	0.51 (±0.55)	0.03 (±0.02)	0.01 (±0.01)	0.008 (±0.004)
800	7.53 (±5.78)	16.84 (±3.84)	20.03 (±2.94)	20.88 (±3.93)	18.45 (±2.69)	16.38 (±2.71)	11.95 (±2.09)	8.71 (±1.69)	4.78 (±2.03)	0.21 (±0.22)	0.06 (±0.08)	0.03 (±0.03)

[a] Mean value for eight volunteers with detectable ketoconazole C_p.
[b] ND, Not detectable.

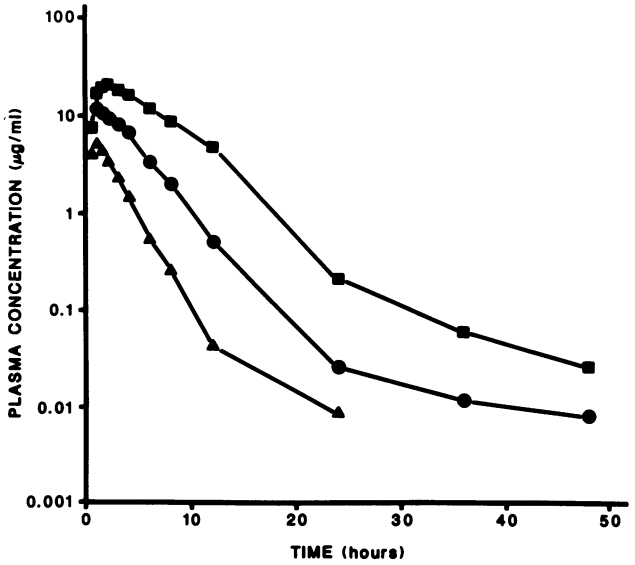

FIG. 2. Mean ketoconazole C_p-t profiles for 12 volunteers after the administration of 200-, 400-, and 800-mg doses. ▲, 200 mg; ●, 400 mg; ■, 800 mg.

an empty stomach. C_{max}s were reached within 2 h after administration. The C_{max} (4.22 μg/ml) found after the administration of the tablet was comparable to those of other studies performed either under fasting conditions (4.1 μg/ml; 9), with meals (3.6 μg/ml; 4), or before meals (3.2 μg/ml; 5). The relative bioavailability of the tablet formulation found in the present study (81%) was slightly higher than that reported earlier (75%) in a similar study of 12 male volunteers (5). In the earlier study, ketoconazole was given to the subjects immediately before meals.

In the present study, the disposition of ketoconazole appeared to follow a multiexponential pattern, and distribution equilibrium was not reached until about 8 to 12 h postadministration. In most of the earlier studies, a single-compartment model was adapted to describe the disposition of ketoconazole in humans (3). This may have been so mainly because less sensitive analytical methods or shorter blood sampling schedules or both were used in the earlier studies. In this study, we monitored the C_p of ketoconazole for up to 48 h with a more sensitive high-performance liquid chromatography assay (detection limit, 2 ng/ml) and found a biphasic decline in the C_p-t profiles, with the values of the initial and terminal $t_{1/2}$ very similar to that reported in an earlier study (5), which used a sensitive gas chromatography method to determine the ketoconazole C_p.

The mean area under the curve from 0 to 12 h was found to contribute more than 95% (tablet, 95.6 [±6.2]%; suspension, 98.0 [±2.5]%) of the total AUC; this suggests that the accumulation of ketoconazole in the body after multiple administration (a regimen of once or twice a day) might be minimal if the superposition principle applies (6). In this regard, it is interesting that 1.5 to 3 h after the daily dose, the ketoconazole C_ps were found to remain relatively stable, in the range of 3 to 5 μg/ml, in 30 patients on a 200-mg/day regimen for 28 weeks (11). Antipyrine clearance also remained relatively constant in healthy subjects after a multiple-dose regimen of ketoconazole (3).

There was wide intersubject variation in the C_p-t profiles observed after the administration of ketoconazole. A similar observation was reported earlier (4). This may reflect the differences in the absorption and disposition kinetics of ketoconazole among the subjects. The greater intersubject variation observed after administration of the tablet formulation (Table 2) may have been due to the fact that the dissolution, and therefore the absorption, of ketoconazole requires a low gastric pH (pH <3) (2) and the fasting gastric pH values vary among subjects (1).

The doses used in our dose proportionality study covered a wider range than in an earlier study (5). The data obtained from 12 subjects who received three ascending solution doses (200, 400, and 800 mg) suggest that dose-dependent kinetics were operative under our study conditions. This conclusion was reached based on the following results. (i) Dose-normalized C_p-t plots were not superimposable. (ii) The increase in the AUC was more than proportional to the increase in the dose. (iii) The semilogarithmic plots of C_ps curved inward at higher dose levels (Fig. 2) (12). These dose-dependent phenomena may be attributed to the first-pass metabolism in the liver or in the gastrointestinal tract or both, which became saturated at higher doses (see the AUC versus dose plot in Fig. 3). A similar observation was made earlier in 12 subjects after the administration of 100-, 200-, and 400-mg tablets (5). After absorption, ketoconazole undergoes extensive hepatic metabolism (5). Ketoconazole is widely distributed and has very high plasma protein binding (99% bound at a drug concentration of 1 μg/ml in an in vitro study with human plasma [7]). Whether there is a capacity-limited hepatic metabolism or a change in distribution pattern of ketoconazole due to a change in tissue or plasma protein binding or both at higher doses remains to be investigated. Better absorption, nonlinear elimination, saturable first-pass metabolism, or a change in the V of ketoconazole have also been suggested as explanations for the

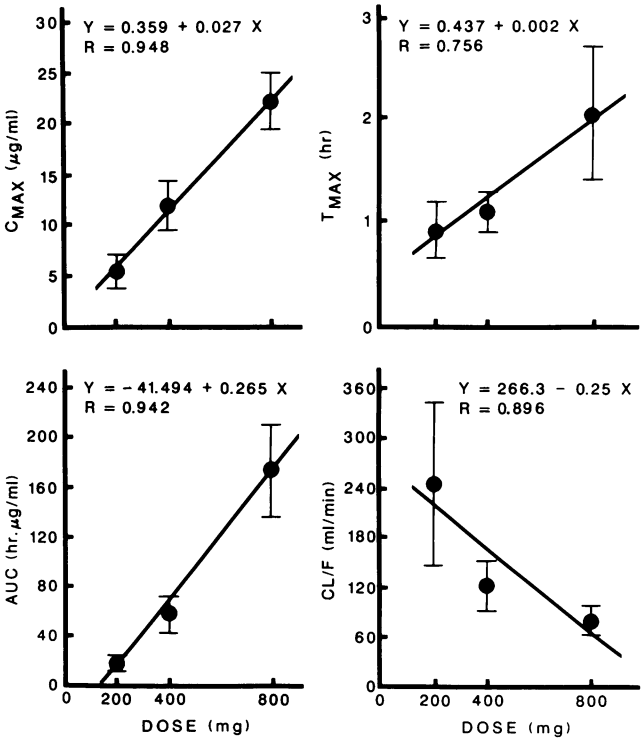

FIG. 3. Correlation of ketoconazole dose with C_{max}, T_{max}, AUC, and CL/F. Bars, ± Standard deviation.

dose-dependent phenomena observed when doses increase from 100 to 400 mg (3).

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