Pharmacokinetics of Itraconazole and Hydroxyitraconazole in Healthy Subjects after Single and Multiple Doses of a Novel Formulation

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Itraconazole is a broad-spectrum antifungal triazole that has been in use for almost two decades for both the prophylaxis and treatment of invasive fungal diseases. Initially, itraconazole was available as an oral agent only. The formulation used initially was an encapsulated form, but the absorption of itraconazole in a subset of immunocompromised patients was not optimal, and its pharmacokinetics varied considerably between patients (3, 10, 16, 18). In addition, absorption was highly influenced by gastric pH and the use of antacids (11, 12), as well as by concomitant food intake (27, 28). An oral solution based on hydroxypropyl-beta-cyclodextrin (HPBCD) became available in the late 1990s. That formulation showed a more favorable pharmacokinetic profile than capsules (4–6, 8, 13, 20, 22, 25) and is superior to capsules in formulation showed a more favorable pharmacokinetic profile than capsules (4–6, 8, 13, 20, 22, 25) and is superior to capsules in

The development of formulations for intravenous use was hampered by the poor solubility of itraconazole in water, but eventually, a 40% HPBCD solution was made available in the United States and elsewhere. However, the presence of large amounts of HPBCD can limit the use of higher doses, even though the dextrin compound is readily eliminated (26) and can be dialyzed (15). This led to the exploration for an alternative parenteral formulation of itraconazole in which larger crystals of the drug substance were milled in the surfactant pluronic F108, generating physically stable dispersions consisting of medium-size crystals (i.e., 50% were <200 nm and 90% were <335 nm). This so-called NanoCrystal formulation (NCF) provided a suitable delivery system for all commonly used routes of administration (14). Studies with several animal species showed that the drug particles were specifically trapped in Kupffer cells in the liver and spleen (Johnson & Johnson Pharmaceutical Research & Development, data on file). This might result in significant changes in the pharmacokinetics of itraconazole as NCF compared to that of the HPBCD formulation. Animal studies indicated that pharmacokinetic changes were related to the size of the particles and were most pronounced for larger crystals (i.e., those ≥340 nm).

The objective of the present studies was to investigate the pharmacokinetics of itraconazole and its hydroxy metabolite after NCF was administered intravenously to healthy subjects. In the first study, the drug was given in escalating doses (single-ascending-dose [SAD] study). In the second study, the drug was given as a single dose and, after a washout of at least 2 weeks, as multiple ascending doses over 7 days (multiple-ascending-dose [MAD] study). In addition, the pharmacokinetics of the HPBCD formulation were determined in a parallel group of subjects to identify any major differences in pharmacokinetics between the two formulations.

MATERIALS AND METHODS

Subjects and samples. Two studies were performed. Study 1 was a SAD study with three planned sequential dose levels of itraconazole as NCF and two groups

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of nine healthy subjects each, with six subjects in each group receiving vero and three subjects in each group receiving placebo. The subjects in group A were scheduled to receive 50, 200, and 400 mg or placebo; and the subjects in group B were scheduled to receive 100, 300, or 500 mg or placebo. Study 2 was a single-MAD and a multiple-MAD study (MAD$_D$ and MAD$_C$) with 100, 200, and 300 mg of itraconazole as NCF and groups of six subjects each, with four subjects in each group receiving vero and two subjects in each group receiving placebo. In addition, one group of four subjects was given 200 mg itraconazole as in the HPBCD formulation. The washout between the single-dose and multiple-dose assessments was at least 2 weeks. Multiple doses were given every 24 h except on days 1 and 2, when the dose was given every 12 h. Both studies were performed as single-center, open-label, randomized, placebo-controlled studies.

Healthy male and female subjects were randomly assigned to receive itraconazole or placebo at each dose level. The itraconazole NCF was administered intravenously over 1 h (SAD study) or as a 1-h infusion (MAD study). The itraconazole HPBCD formulation was administered as a 1-h infusion (single dose) or a 2-h infusion (multiple doses). Subjects fasted overnight for at least 10 h before and for 2 h after dosing on the day of the full pharmacokinetic analysis. Venous blood samples were taken from an indwelling cannula from the arm opposite that used to infuse the itraconazole. In the single-dose studies, samples were collected in heparinized tubes immediately before the start of the infusion; at 0.5, 1, and 2 h during the infusion, when applicable; at 0, 0.08, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, and 8 h after the end of the infusion; and at 24, 32, 72, 96, and 168 h after the start of the infusion. In the multiple-dose study, samples were collected just before and 1 h after the infusion on days 1 to 7. On the 7th day, additional blood samples were taken at times similar to those used in the single-dose studies. The blood samples were allowed to remain undisturbed at room temperature for at least 2 h to ensure the complete dissolution of the itraconazole NCF and minimal bioanalytical variability in these early clinical studies. Unpublished research and pharmacokinetic modeling at Johnson & Johnson indicate that nanocrystals rapidly release itraconazole, with half-lives of 9 and 2 min in human and dog plasma, respectively. The blood samples were then centrifuged at 1,000 g for 10 min, and the resulting plasma was transferred into polypropylene tubes and stored at −20°C until analysis. Both studies were conducted in full compliance with good clinical practice guidelines and with prior ethics committee approval.

Analytical procedure. Itraconazole and hydroxyitraconazole concentrations were measured by high-performance liquid chromatography, as described previously (24). The assay of itraconazole in plasma had lower limits of quantification (LOQ) of 2 and 5 μg/liter. The calibration curve ranged from 2 to 5,000 μg/liter. The LOQ applies to the concentration in plasma obtained after the blood is left for 2 h at room temperature, followed by centrifugation. The accuracy of the assay ranged from 97.1% to 98.7% for independently prepared itraconazole quality control samples with concentrations ranging between 13.8 and 717 μg/liter and from 94.7% to 99.5% for hydroxyitraconazole control samples with concentrations ranging between 14.5 and 753 μg/liter. The precisions (coefficients of variation) ranged between 1.0% and 3.4% for itraconazole and between 1.4% and 3.1% for hydroxyitraconazole.

Pharmacokinetic and statistical analyses. Pharmacokinetic parameters were derived by noncompartmental analysis. The area under the concentration-time curve (AUC) from time zero to infinity (AUC$_{0\rightarrow\infty}$) was determined by use of the linear trapezoidal rule with extrapolation to infinity by using the elimination rate constant. The elimination rate constant ($\lambda_e$) was determined by linear regression of the terminal points of the log-linear plasma concentration-time curve. The terminal half-life was defined as 0.693/$\lambda_e$. Clearance (CL) was calculated as dose/AUC$_{0\rightarrow\infty}$ or as dose/AUC$_{144\rightarrow168}$ (where AUC$_{144\rightarrow168}$ is the AUC from 144 to 168 h) and the volume of distribution ($V$) as dose/(AUC$_{144\rightarrow168}$ $\lambda_e$) (21).

Differences in terminal half-lives were determined from the linear regressions of log-transformed concentrations. Statistical analysis was performed by using SAS software for nonparametric or parametric procedures when applicable. A two-sided $P$ value of $<0.05$ was considered statistically significant.

RESULTS

Subject characteristics, treatment compliance, and safety assessments. The demographic data for the subjects randomized in the studies and receiving vero are shown in Table 1. There were no significant differences in the characteristics between the subjects in the different dose groups in either of the two studies. In the SAD study the only adverse events reported were in two subjects receiving 300 mg itraconazole as NCF, who developed acute severe localized back pain shortly after the start of the infusion, accompanied by lumbar muscular spasms. These events led to the discontinuation of the infusion. Treatment at the 300-mg level and higher was therefore suspended. No clinically relevant abnormalities in laboratory or cardiovascular measurements were observed. It was concluded that these events were related to the relative high infusion rate at the higher dose levels. Consequently, the infusion time for the MAD study was prolonged to 2 h and 3 h for the 200- and 300-mg doses, respectively; and no further localized back pain or lumbar muscular spasms were reported. Except for these events, treatment compliance was 100% in both studies. In the MAD study, several subjects reported mild to moderate local reactions at the site of infusion (swelling, redness, pain, phlebitis), and these were approximately equally divided over all treatments, including the placebo; headache and dizziness, known adverse reactions to itraconazole, were reported by two subjects each.
However, this was entirely due to the relatively low AUC0–24/H11009 at the 50-mg dose level; there was no significant increase over the 100- to 300-mg range. For hydroxyitraconazole there was no difference in the dose-normalized peak concentrations in plasma between the dose groups.

Multiple-ascending-dose study. Itraconazole and its metabolite, hydroxyitraconazole, could not be detected in any plasma sample before the administration of itraconazole at each dosing phase, indicating that a minimum of 2 weeks of washout between both phases was sufficient to prevent the carryover of itraconazole and hydroxyitraconazole in plasma. Figure 3 shows the concentration-time plots for itraconazole and hydroxyitraconazole for the 100- and 300-mg doses of itraconazole as NCF. Table 3 shows the mean estimated pharmacokinetic parameter values for itraconazole and hydroxyitraconazole for the three NCF treatment groups and the group receiving the HPBCD formulation. The results indicate that there was no accumulation of itraconazole over the treatment period studied once steady state is achieved, which is after approximately 48 to 72 h, while the steady state of the metabolite was achieved after only 1 week of treatment. The pharmacokinetic parameter values at steady state in the MAD study differed from those obtained in the SAD study. The AUCs were higher, and in particular, the half-life was associated with a dose-dependent increase. This was also true for hydroxyitraconazole. This is more apparent in Fig. 4, which shows the terminal-phase concentration-time profile of itraconazole during the MAD study for the three dose levels. It is evident that the half-life was increased at increasing dose levels, which was statistically significant (P < 0.001 for both itraconazole and hydroxyitraconazole).

**FIG. 2.** Dose (D) proportionality plots of itraconazole. The regression line for the single-dose studies is based on a fit to the data of both studies (SAD and MADs studies).

Comparison of NCF and HPBCD formulation. Comparison of the 200-mg doses for the two formulations showed that the mean peak concentrations in plasma during the infusion of NCF were substantially higher than those during the infusion of the HPBCD formulation (Fig. 5). However, during the first 30 min after the infusion of NCF, there was a more rapid

![Graphs showing concentration-time plots for itraconazole and hydroxyitraconazole](image)

**FIG. 1.** Concentration-time plots of itraconazole (upper panel) and hydroxyitraconazole (lower panel) after single doses of 50, 100, 200, and 300 mg of NCF. Each group except for the 300-mg group had six subjects; the 300-mg group had two subjects.

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th><strong>SAD study</strong></th>
<th></th>
<th><strong>MADs study</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC0–24/H11009 (µg · h/liter)</td>
<td>λ1 (1/h)</td>
<td>Terminal half-life (h)</td>
</tr>
<tr>
<td>50</td>
<td>2,506 (296)</td>
<td>0.0238 (0.0028)</td>
<td>29.5 (3.5)</td>
</tr>
<tr>
<td>100</td>
<td>7,498 (2,697)</td>
<td>0.0266 (0.0053)</td>
<td>26.8 (4.5)</td>
</tr>
<tr>
<td>200</td>
<td>15,292 (2,869)</td>
<td>0.0243 (0.0059)</td>
<td>29.7 (5.6)</td>
</tr>
<tr>
<td>300</td>
<td>28,146 (1,488)</td>
<td>0.026 (0.0075)</td>
<td>27.8 (8.1)</td>
</tr>
</tbody>
</table>

*Values are means (standard deviations). n = 6 per group for active treatment in the SAD study except for the 300-mg dose (n = 2); n = 4 per active treatment group in the MAD study.*
decline, and as a consequence, the difference between the two formulations for the other pharmacokinetic parameters was less pronounced or was not significant at all. Both formulations were comparable with respect to the terminal half-life, both after a single dose (results not shown) and during steady state (Table 3).

**DISCUSSION**

We describe the pharmacokinetics of itraconazole and hydroxyitraconazole after single and multiple ascending doses of the NCF. We also compared the pharmacokinetics of NCF to that of the HPBCD formulation for one dose level. Each study group comprised a number of subjects receiving placebo to determine whether there were relevant and significant side effects. Although the study design was initially double blinded in that respect, the appearance of the placebo formulation was different from that of the itraconazole formulation and blinding could not be achieved. Thus, both studies were essentially open label. Itraconazole was well tolerated except in the SAD study at the higher dose of 300 mg, where two subjects complained of severe localized back pain immediately after infusion. This was thought to be related to the duration of infusion, and subsequently, the infusion time was restricted to 100 mg per hour. No clinically relevant side effects were observed by using the prolonged infusion time at higher doses up to 300

![Concentration-time plots of itraconazole (upper panel) and hydroxyitraconazole (lower panel) after multiple doses of 100 mg or 300 mg of NCF. For each dose level, peak and trough concentrations were determined on each study day.](image-url)
mg, and we conclude that the initial hypothesis that the infusion time was too short for the higher dose was plausible.

Although the terminal half-life of itraconazole and, in particular, that of hydroxyitraconazole were somewhat longer after the administration of NCF than those after the administration of the HPBCD formulation, the differences were not significant. One might therefore conclude that the formulation itself has no significant effect on the elimination of the drug. However, there were only few subjects in each group, with wide variation in the half-lives between them. Since the study was not designed to compare the two formulations but, rather, was designed to describe the pharmacokinetic profile of NCF, a more extensive study is required to be conclusive in this respect. The $\text{AUC}_{0-\infty}$ values at steady state were comparable between the two formulations.

The steady-state concentrations of itraconazole were reached after 48 h (four doses) during both treatments, while for hydroxyitraconazole, 7 days was needed to reach steady state. This is in agreement with the findings of earlier pharmacokinetic studies (6, 7, 23), which documented trough concentrations over a 7-day treatment with the HPBCD formulation in various patient groups. The concentrations in those studies were slightly lower, however, with mean trough levels between 316 and 620 $\mu$g/liter after 48 h and 337 to 644 $\mu$g/liter after 7 days of treatment, whereas the trough levels were 916 $\mu$g/liter after 7 days of treatment with the HPBCD formulation in the current study. The 200-mg dose of itraconazole as NCF resulted in even higher mean trough concentrations (1,249 $\mu$g/liter). Similarly, the concentrations of hydroxyitraconazole were higher than those reported in the previous studies. This might simply be an artifact related to the small sample size in the current studies or might be due to the fact that the current studies were performed with healthy subjects, while the other studies were performed with various patient groups. The fraction of unbound drug is known to be smaller (1, 2) in healthy subjects, and renal elimination may thus be slower.

The mean maximum plasma itraconazole concentrations at the end of the infusion of NCF were 1,410, 3,510, and 6,220 $\mu$g/liter for doses of 50, 100, and 200 mg, respectively, and were considerably higher than that reported earlier for a single dose of 100 mg itraconazole as the HPBCD formulation, which resulted in a mean plasma concentration of 660 $\mu$g/liter at the end of infusion (Johnson & Johnson Pharmaceutical Research & Development, data on file). This difference, if it is indeed true, could be explained by assuming that during infusion not all itraconazole particles are dissolved. Nondissolved itraconazole is trapped in plasma and is therefore not available for diffusion and distribution to the peripheral tissues, resulting in higher concentrations in plasma. Another explanation could be that the particles are taken up by the mononuclear phagocyte system through opsonization. However, itraconazole in NCF dissolves relatively quickly, as can be concluded from the observation that peak concentrations in plasma were reached before the end of infusion, when there was equilibrium between the

![FIG. 4. Terminal-phase concentration-time profiles of itraconazole during the MAD study for three dose levels: 100, 200, and 300 mg. The slopes of the three regression lines are significantly different.](http://aac.asm.org/)

![FIG. 5. Concentration-time plots of itraconazole (upper panel) and hydroxyitraconazole (lower panel) after multiple doses of 200 mg as NCF (●) or the HPBCD formulation (□). For each dose level, peak and trough concentrations were determined on each study day.](http://aac.asm.org/)
rate of infusion and the rate of dissolution of the drug particles in blood.

The pharmacokinetics of itraconazole after the infusion of single doses in the SAD and the MAD studies were slightly more than dose proportional over the range of 50 to 300 mg in a combined analysis. However, this seems to be entirely due to the relatively low AUCs at the 50-mg dose level. One explanation could be the lower level of quantification reached relatively earlier at the low dose than at the higher doses and therefore resulted in a slight underestimation of AUC0–24. On the other hand, a more-than-dose-proportional increase in AUC0–24 does fit with the longer half-life observed after higher doses during the MAD study (Fig. 4). Surprisingly, the dose-normalized AUC0–24 for the 100-mg dose compared to that for the 300-mg dose appeared to be higher for hydroxyitraconazole as well, indicating that conversion from itraconazole to the hydroxy metabolite is not the rate-limiting step in this case but must be sought in the elimination of both components. One explanation could be the formulation itself, as explained above, whereby the drug particles take some time to dissolve in blood. At higher concentrations, this process might take slightly longer, if the crystals are, as indicated in animal studies, trapped in liver and spleen cells, which may then act as a deep compartment, thereby explaining the significant longer half-lives at higher doses.

In clinical studies, it assumed that a trough value of at least 500 μg/liter itraconazole is required to successfully prevent or treat invasive fungal disease (17, 19), which both formulations achieve with doses of 200 mg or higher. Moreover, the variance in trough levels of the NCF of itraconazole was relatively low, which is in contrast to what is observed after oral treatment. The wide variability for oral itraconazole is probably due to variation in bioavailability after oral dosing, indicating the necessity of starting treatment of high-risk patients intravenously. As with the HPBCD formulation, such trough levels of at least 500 μg/liter itraconazole can readily be achieved by using the NCF of itraconazole, which may be a viable alternative to the existing HPBCD formulation for the further optimization of antifungal treatment.

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