Influence of grapefruit juice on itraconazole plasma levels in mice and guinea pigs

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Levels of itraconazole and its metabolite hydroxy-itraconazole were measured by HPLC in plasma samples from guinea pigs and DBA/2 and BALB/c mice after oral or intraperitoneal treatment with itraconazole/cyclodextrin solution at 5 and 20 mg/kg daily for 7 days. The animals were randomly assigned to receive grapefruit juice or drinking water in their fluid bottles, since components of grapefruit juice have been found to alter the pharmacokinetic behaviour of pharmaceuticals that interact with cytochrome P450 enzymes. The results demonstrated clear differences in itraconazole pharmacokinetics between the three animal types studied. The total levels of antifungally active azole were highest in DBA/2 mice and lowest in guinea pigs, regardless of the route of administration. Guinea pigs that drank grapefruit juice had higher plasma levels of azoles after oral administration of the drug, but the juice did not influence levels of itraconazole or its metabolite in guinea pigs treated intraperitoneally, or in either mouse strain treated orally or intraperitoneally. This result indicates that grapefruit juice effects on the pharmacokinetics of itraconazole are species dependent and confined to the gastrointestinal tract, influencing drug absorption. Differences in itraconazole pharmacokinetics between mouse strains need to be considered in the design of experiments involving itraconazole treatment of mice experimentally infected with fungi.

Introduction

Itraconazole is a broad-spectrum antifungal agent used clinically in a variety of serious fungal infections in normal and immunocompromised hosts, including aspergillosis, blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis and disseminated *Penicillium marneffei* infections.1-4 For many years, the compound was available only in the form of oral capsules, whose bioavailability varies between patients.5 A new solution of itraconazole in hydroxypropyl-β-cyclodextrin was recently introduced for intravenous infusion. This preparation ensures favourable plasma levels of itraconazole and its antifungally active metabolite hydroxy-itraconazole (HO-itraconazole).6-9

In experiments on survival of mice with disseminated fungal infections treated prophylactically or therapeutically with itraconazole, doses required to show any enhancement of survival have usually been extremely high—commonly >40 mg/kg,10-18 with some authors unable to demonstrate effects even at 100 mg/kg.19-21 In contrast, when guinea pigs have been used as hosts for experimental disseminated fungal infections, orally and parenterally administered itraconazole has significantly enhanced survival at doses of 2.5–5 mg/kg.22-24 These regimens are much closer to those used in humans, where itraconazole is normally given in doses equivalent to 4–8 mg/kg in a 50 kg subject.

There is clearly a discrepancy between the efficacy of itraconazole in mouse and guinea pig models of systemic mycoses, and pharmacokinetic differences in the behaviour of itraconazole between species would normally be assumed to be a likely source of the difference. However, on the few occasions when they have been determined, reported plasma levels of itraconazole in mice have shown high variability and inconsistency. Estimates of peak levels of the drug include <1 mg/L after a 200 mg/kg oral dose and 7 mg/L after a 20 mg/kg oral dose for polyethylene glycol formulations;10,25 and 7 and

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14.5 mg/L after 25 mg/kg oral doses formulated in cyclodextrin. These concentrations were determined by itraconazole bioassays, and their variability may therefore reflect either the inherent imprecision of bioassays and their tendency to overestimate itraconazole levels, or a variability between mouse strains that has not previously been investigated. Peak plasma levels of itraconazole in guinea pigs have barely exceeded 1 mg/L (measured by HPLC) even when the agent is administered intravenously. The persistence of itraconazole in the blood of mice and guinea pigs is essentially unknown.

Sugar & Liu showed that plasma levels of voriconazole, normally negligible after oral administration in mice, were raised when the mice received grapefruit juice in place of water to drink. Also, the use of grapefruit juice to boost voriconazole levels demonstrated the efficacy of orally administered voriconazole against experimental murine blastomycosis. Grapefruit juice contains a bioflavonoid compound, naringin, and a furanocoumarin, bergamottin, both of which inhibit the cytochrome P450 enzyme CYP3A4 in the human intestine and therefore increase blood levels of drugs that are normally metabolized by this enzyme. In humans given grapefruit juice concomitantly with itraconazole, a decrease in itraconazole plasma levels and their duration was observed. However, it remains possible that in mice, grapefruit juice may boost itraconazole levels in the same way as it boosts voriconazole levels, and thus it may provide a pharmacokinetically preferable model for the evaluation of itraconazole effects in vivo.

The present study was undertaken to provide a fuller characterization of the pharmacokinetic behaviour of itraconazole given orally and intraperitoneally (ip) to guinea pigs and to two strains of mice, and to see whether grapefruit juice substituted for drinking water led to improved itraconazole pharmacokinetics. Plasma levels of itraconazole and HO-itraconazole were determined by HPLC, and two mouse strains were included in the study to investigate possible variations in itraconazole pharmacokinetics between them.

Materials and methods

Animals

The experiments were approved by the local ethics committee and conformed to the terms of UK Home Office licences for research on animals. Female DBA/2 and BALB/c mice (Harlan) with a weight range of 17–23 g and male guinea pigs with a weight range of 300–450 g were maintained under conditions specified by the Health and Safety Executive for level 2 biohazard containment. The animals were supplied with food ad libitum. After a 2 week period of acclimatization after delivery, half of the animals of each species and strain were randomized to receive Del Monte brand grapefruit juice, purchased from the local supermarket. Consumption of drinking fluid was monitored daily.

Itraconazole and other compounds

Itraconazole in cyclodextrin for injection was provided by Ortho Biotech Products, L.P., Raritan, NJ, USA. Sterile 40% hydroxypropyl-β-cyclodextrin (cyclodextrin) for injection was provided by the Janssen Research Foundation, Beerse, Belgium. Samples of the itraconazole solution were diluted in 40% cyclodextrin under full aseptic precautions to provide concentrations suitable for administration to the animals. For itraconazole assays, pure powders of itraconazole, HO-itraconazole and R051012 (HPLC internal standard) were supplied by the Janssen Research Foundation.

Experimental design

Three days after grapefruit juice had been substituted for drinking water for half of the experimental animals, treatment of all animals began with itraconazole solution or cyclodextrin placebo. Animals receiving itraconazole were dosed orally or ip at 5 or 20 mg/kg daily for 7 days. Blood samples were obtained from two animals at 2 and 7 h after itraconazole or placebo was given on days 1, 3 and 7. For guinea pigs, the blood was obtained from the marginal ear vein; for mice, the animals were killed and blood was removed by cardiac puncture. This design required six mice per treatment group and four guinea pigs per treatment group, each sampled on three occasions. Blood samples were collected in citrated tubes, centrifuged and the plasma supernatants stored at –20°C until assay. Antifungal concentrations were determined by HPLC for all samples.

HPLC for itraconazole and HO-itraconazole

Itraconazole and HO-itraconazole concentrations were determined with a 10 cm × 4.6 mm ID column packed with 3 μm BDS-C18 Hypersil (Alltech Associates) at 30°C in an Agilent series 1100 apparatus with an ultraviolet spectrophotometric detector set at 263 nm. All solvents were ‘HPLC grade’ (Fisher Scientific, UK) and the extraction procedure was carried out in 1.5 mL capped plastic microcentrifuge tubes. The eluent was a 35:65 (v/v) mixture of 0.01 M ammonium acetate and acetonitrile, run at 0.8 mL/min.

Samples (100 μL) of test plasma, or reference samples spiked with known concentrations of itraconazole or HO-itraconazole prepared in methanol and evaporated to dryness before the addition of pooled human plasma, were mixed with 300 μL of R051012 (internal standard) at 10 mg/L in methanol. The mixtures were vortexed for 30 s then centrifuged at full speed for 10 min in a bench microcentrifuge. The supernatant fluids were evaporated to dryness at 60°C then redissolved in 100 μL of eluent. Sample volumes of 40 μL were
applied to the column. Concentrations of itraconazole and HO-itraconazole in samples of plasma were calculated from ratios of peak areas for each compound to that of the internal standard by reference to a calibration curve derived from freshly spiked reference samples in each run. The assays were linear for the detection of itraconazole and HO-itraconazole concentrations from 16 ng to 16 µg per 100 µL plasma sample. Coefficients of variation over this range were from 16.6% to 0.2%.

Because itraconazole and HO-itraconazole have equipotent antifungal activity in vitro, statistical comparisons were made on total (itraconazole + HO-itraconazole) plasma concentrations.

Results

Consumption of water and grapefruit juice

The mean fluid consumption for the three animal types studied was calculated from daily measurements over the period of the experiments as follows. For animals given water, DBA/2 mice consumed 5.5 ± 2.5 mL, BALB/c mice consumed 5.1 ± 1.5 mL and guinea pigs consumed 91 ± 26 mL daily. The corresponding volumes for animals given grapefruit juice were 3.2 ± 1.6, 4.0 ± 1.4 and 71 ± 22 mL. The data therefore show a highly significant reduction in fluid intake for all animals given grapefruit juice in place of water to drink (Student’s t-test, P < 0.01).

Plasma concentrations of itraconazole and HO-itraconazole

None of the plasma samples from placebo-treated animals contained detectable itraconazole or HO-itraconazole. Figure 1 summarizes the results of the azole plasma level determinations from animals that received itraconazole dosed at 5 or 20 mg/kg, orally or ip. In all three animal types, the plasma levels of itraconazole and HO-itraconazole were dose related, with higher levels measured for animals treated with 20 mg/kg than for animals treated with 5 mg/kg. For all three types the common trend was for itraconazole levels to decrease and for HO-itraconazole levels to increase between 2 and 7 h after the dose.

Plasma from guinea pigs given water and treated orally with itraconazole at 5 mg/kg showed detectable levels only of HO-itraconazole and only in a single sample on day 1 at 2 h after the dose (Figure 1); itraconazole was not detected at any time. Among the equivalent guinea pigs given grapefruit juice to drink, only plasma taken 2 h after the dose on day 7 contained detectable levels of itraconazole and HO-itraconazole. For the comparable groups of guinea pigs treated at 20 mg/kg orally, the data showed detectable levels of itraconazole and HO-itraconazole in most samples. However, total azole levels in the animals drinking grapefruit juice were generally higher, commonly four-fold higher, than in those drinking water (Table 1). The total azole levels in the ‘grapefruit juice’ group treated orally with itraconazole were significantly higher than in the ‘water’ group (Wilcoxon ranked sign test, P < 0.05). The highest peak levels of the azoles were measured in samples taken on day 3; they were lower in the plasma samples taken after 7 days of daily oral dosing (Figure 1).

For guinea pigs treated ip with itraconazole at 5 mg/kg, plasma levels of itraconazole and HO-itraconazole were higher regardless of the drinking fluid supplied than in the orally treated animals (Figure 1). There was no significant difference between the total azole levels for ip-treated animals in the grapefruit juice and water groups (Table 1). By day 7 of ip treatment, plasma levels of the azoles were low or undetectable in the guinea pigs given drinking water; they were of a similar magnitude throughout the 7 day dosing period in the animals drinking grapefruit juice. This difference on day 7 was the only one observed between the juice and water groups. The percentage of HO-itraconazole in the total azole concentration was higher at 7 than at 2 h on only seven of 15 measurable occasions in guinea pigs, suggesting a

![Figure 1. Plasma concentrations of itraconazole (open symbols) and HO-itraconazole (filled symbols) in animals treated with itraconazole at 5 mg/kg (dashed lines) or 20 mg/kg (solid lines). The animals were given water (circles) or grapefruit juice (squares) to drink. (a) Guinea pigs, oral dosing; (b) guinea pigs, ip dosing; (c) DBA/2 mice, oral dosing; (d) DBA/2 mice, ip dosing; (e) BALB/c mice, oral dosing; (f) BALB/c mice, ip dosing.](image-url)
leisurely rate of conversion of itraconazole into its metabolite in these animals.

The data for mice indicated species- and strain-specific differences in plasma azole levels. For both mouse strains, total azole levels after 7 days of dosing, whether ip or oral, were not lower than at earlier times, whereas there was a tendency among the guinea pig samples towards lower plasma levels on day 7 than on days 1 and 3 (Table 1). In BALB/c mice, peak levels of itraconazole were considerably higher by day 7 than on days 1 and 3; this effect was not apparent for DBA/2 mice (Figure 1). For both mouse strains, rates of conversion of itraconazole into HO-itraconazole were higher than in guinea pigs, with HO-itraconazole percentages of total azole at 7 h higher than at 2 h on 20 of 23 measurable occasions (DBA/2 mice) and 11/15 measurable occasions (BALB/c mice). For DBA/2 mice, the differences between total azole levels were not statistically significant for the orally treated versus the ip-treated animals; however, this difference was statistically significant (Wilcoxon signed rank test, \( P < 0.05 \)) for the BALB/c mice treated at 20 mg/kg (Table 1). For both mouse strains there were no significant differences in levels between mice given water and those given grapefruit juice to drink.

For DBA/2 mice, the total azole levels at most sample points were significantly higher than those from the corresponding guinea pigs and for BALB/c mice (Wilcoxon ranked sign test, \( P < 0.0001 \) for both comparisons; see Table 1). For BALB/c mice, levels were higher than in guinea pigs only for animals treated ip with itraconazole at 20 mg/kg. Overall, plasma concentrations in BALB/c mice were not significantly different from those for corresponding guinea pigs (Wilcoxon ranked sign test, \( P = 0.43 \)).

**Discussion**

This study is, to our knowledge, the largest ever undertaken of itraconazole and HO-itraconazole plasma levels in experi-
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mental animals. It has shown that clear differences exist in the absorption, metabolism and elimination of this antifungal agent between the three animal types studied. Overall, azole plasma levels in DBA/2 mice were significantly higher than those in guinea pigs and BALB/c mice, suggesting a slower rate of elimination of itraconazole and its metabolite in this species. Had the higher levels been apparent only in the orally treated DBA/2 mice, the data would have indicated a higher rate of absorption of the drug; however, the fact that the levels were also significantly higher in DBA/2 mice treated ip with itraconazole points to slower elimination as the explanation. After 7 days of treatment, peak (2 h) levels of itraconazole were much higher in BALB/c mice than in DBA/2 mice or guinea pigs.

Absorption of itraconazole after oral administration was enhanced in guinea pigs given grapefruit juice to drink in place of water. No influence of grapefruit juice was found among ip-treated guinea pigs or among orally or ip-treated mice of either strain. Sugar & Liu²⁹ found that substituting grapefruit juice for drinking water with mice (ICR strain) led to very high plasma levels of voriconazole after the drug was given by mouth, compared with undetectable levels in animals that drank water. If the mechanism for this pharmacological action of grapefruit juice in any way involved the inhibition of cytochrome P450 enzymes in mouse liver, similar effects would have been anticipated in our experiments in all animal species and with ip, as well as oral, administration. Unlike Sugar & Liu,²⁹ we found that all our animals drank smaller volumes of grapefruit juice than of water, suggesting that it was relatively unpalatable to them.

Since we saw a grapefruit juice effect only in orally treated guinea pigs, we conclude that the action of those components of grapefruit juice that inhibit P450 enzymes is confined to the gastrointestinal tract, as has been shown for humans.³² P450 enzymes are located in the intestine not the stomach. We therefore suggest that our negative data for grapefruit juice effects in mice, compared with those for voriconazole,²⁹ indicate either that absorption of itraconazole by mice must occur primarily in the stomach, whereas voriconazole is absorbed in the mouse intestine, or that the mouse strains we used have low intestinal P450 activity compared with the ICR mice used by Sugar & Liu.²⁹ It is unusual for the stomach to be the main site for absorption of a drug, but the data on enhancement of itraconazole bioavailability in humans by manipulation of gastric pH strongly suggest that this agent may indeed be preferentially absorbed from the stomach.³⁵–³⁷

With samples from only two animals for each treatment group and only two sample times for each day when blood was taken, it was not possible to provide a conventional pharmacokinetic profile for itraconazole in small rodents, with determinations of area under the curve and other parameters. The experiments were designed to reveal differences between itraconazole and HO-itraconazole plasma levels in a range of animal types and treatment regimens. However, it is clear that, in DBA/2 mice, plasma levels are high and remain high up to 7 h after dosing. Our plasma level findings with this mouse strain parallel those of others who treated mice with oral itraconazole formulated in cyclodextrin.⁵,²⁶ However, in BALB/c mice the lower plasma levels of azole found, particularly after oral or ip administration of the drug at 5 mg/kg, indicate considerable strain to strain variation in murine pharmacokinetic behaviour of itraconazole.

There remains the paradox, outlined in the Introduction, that therapeutic success with itraconazole in experimental deep-tissue Candida and Aspergillus infections has been poor in mouse models and excellent in guinea pig models. These findings appear to be independent of itraconazole (and HO-itraconazole) serum levels, which are low in guinea pigs, as confirmed by the present study. Experiments to compare the therapeutic activity of itraconazole in different mouse strains, such as the two used in our study, may help to explain the paradox.

Acknowledgements

We are grateful to Amanda Davidson, Marlene Arthur and Steve MacBain for technical assistance. This study was supported by a grant from Ortho Biotech Products, L.P.

References


