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Effect of Cyclodextrin on the Pharmacology of Antifungal Oral Azoles

JOHN S. HOSTETLER,[1,2,3]* LINDA H. HANSON,[3] AND DAVID A. STEVENS[1,2,3]

Division of Infectious Diseases, Department of Medicine, Santa Clara Valley Medical Center, 751 South Bascom Avenue,[1,2] and the California Institute for Medical Research,[3] San Jose, California 95128, and Division of Infectious Diseases, Department of Medicine, Stanford University School of Medicine, Stanford, California 94305[1,2]

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Concentrations of oral azoles in serum were compared in a single-dose pharmacologic study in mice. When hydroxypropyl-β-cyclodextrin was used as a carrier and compared with a standard carrier, polyethylene glycol, drug concentrations determined by bioassay showed that the peak concentration and area under the concentration-time curve were greatly enhanced for itraconazole and saperconazole; moderately enhanced for ketoconazole; but negligibly affected for fluconazole, miconazole, and SCH 42427.

Treatment options for cutaneous and systemic fungal infections have been greatly expanded with the advent of the oral azoles such as ketoconazole and fluconazole. These drugs have shown minimal toxicity, while they have demonstrated efficacy against many invasive fungal pathogens. A new agent, itraconazole, has also shown great promise in this regard.

However, concentrations of certain azoles, such as itraconazole, in serum may be variable and suboptimal, thereby potentially compromising therapy. Indeed, treatment failures have been associated with low concentrations of itraconazole in blood in some patients (3).

Improvement of the oral bioavailability of itraconazole has been problematic. Itraconazole can be solubilized only under extremely acidic conditions, although its absorption is enhanced by the concurrent administration of food (10). Similarly, ketoconazole absorption is markedly inhibited by agents which increase gastric pH (1). In addition to gastric pH, the water solubility of azoles may be important in intestinal tract absorption. Itraconazole, ketoconazole, saperconazole, and miconazole have poor aqueous solubilities because of their hydrophobic structures. This may adversely affect concentrations in blood when the drugs are given orally.

Naturally occurring β-cyclodextrin is a cyclic oligosaccharide of seven glucose units which is a product of the enzymatic degradation of starch by *Bacillus macerans*. The physicochemical properties of cyclodextrins are particularly well suited for utilization as carrier molecules of lipophilic drugs. Their structures are analogous to a truncated cone with a hydrophobic interior and hydrophilic exterior (Fig. 1) (6). This allows the molecular encapsulation of hydrophobic portions of guest molecules, thus shielding them from the polar forces of aqueous solutions. The 0.78-nm internal diameter of β-cyclodextrin is comparable to the dimensions of substituted phenyl groups which contribute to the lipophilicity of itraconazole and other azoles.

The usefulness of natural cyclodextrins has been limited by relatively low aqeous solubility. As a result, cyclodextrins have been substituted at the hydroxyl groups to alter

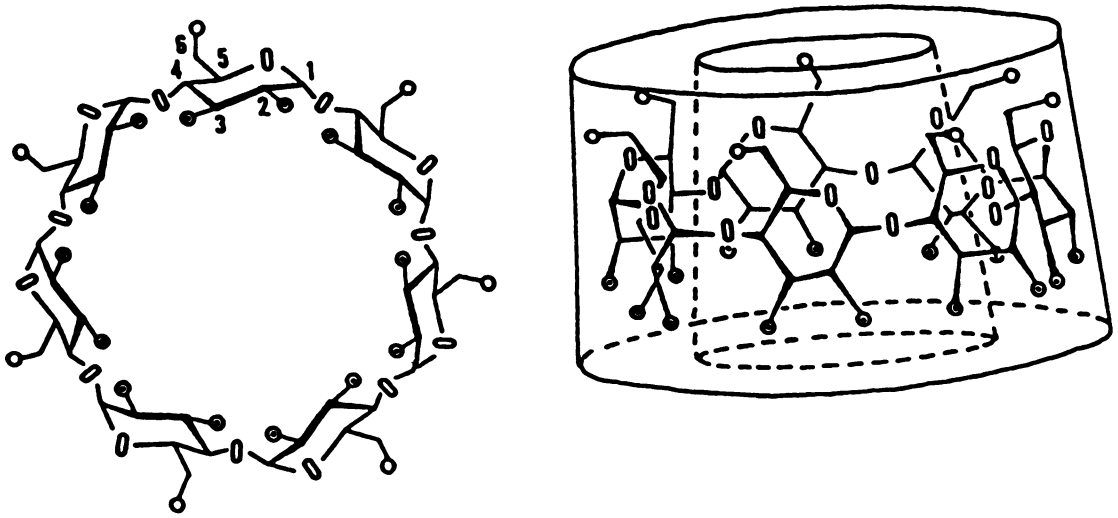

FIG. 1. Diagram of β-cyclodextrin molecule. Reprinted from *Controlled Drug Delivery* (6) with permission of the publisher.

* Corresponding author.

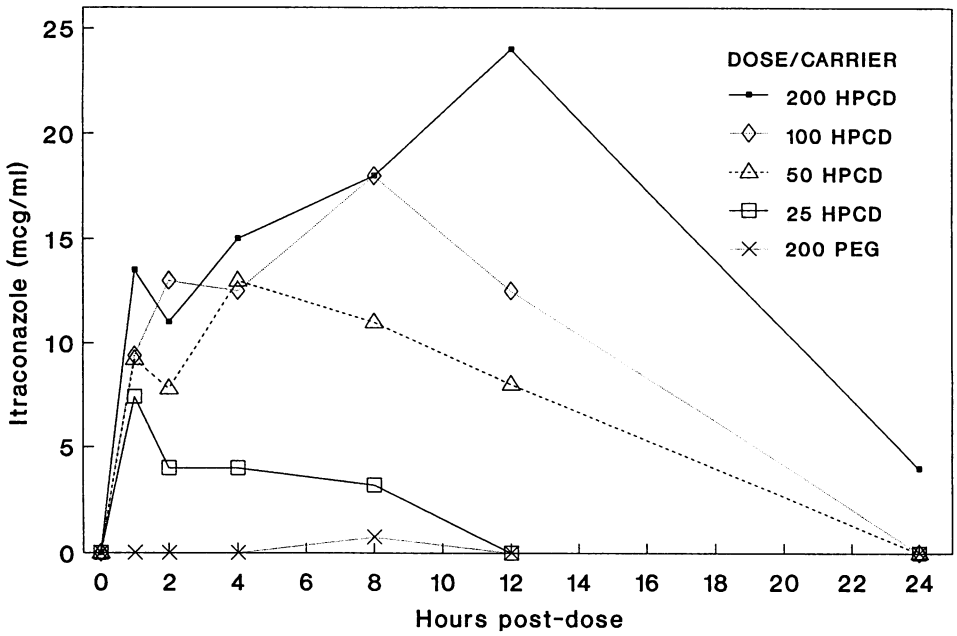

FIG. 2. Itraconazole concentrations over time derived from combined sera of two mice at each time point after a single dose. Drug was solubilized in either HPCD or PEG. Doses are milligrams of itraconazole per kilogram.

both solubility and inclusion characteristics. Hydroxypropyl-β-cyclodextrin (HPCD) demonstrates improved solubility over β-cyclodextrin while maintaining negligible toxicity with oral administration (9).

We compared the effect of HPCD versus that of polyethylene glycol 200 (PEG) as carriers on the pharmacology of various azoles, including itraconazole and ketoconazole (both Janssen Pharmaceutica), saperconzaole (Ortho, Raritan, N.J.), fluconazole (Roerig-Pfizer, New York, N.Y.), and SCH 42427 (Schering-Plough, Kenilworth, N.J.) in a single-dose murine model.

Drugs were solubilized in HPCD (gift of Janssen Pharmaceutica) to make a 100-ml solution at 25 mg/ml according to a protocol that was slightly modified from that provided by Janssen Pharmaceutica. A total of 10 ml of propylene glycol and 0.95 ml of 10 N HCl were combined in a glass beaker with a magnetic stirring bar. The solution was heated to 40 to 50°C in a water bath. Azole powder (2.5 g) was added and the

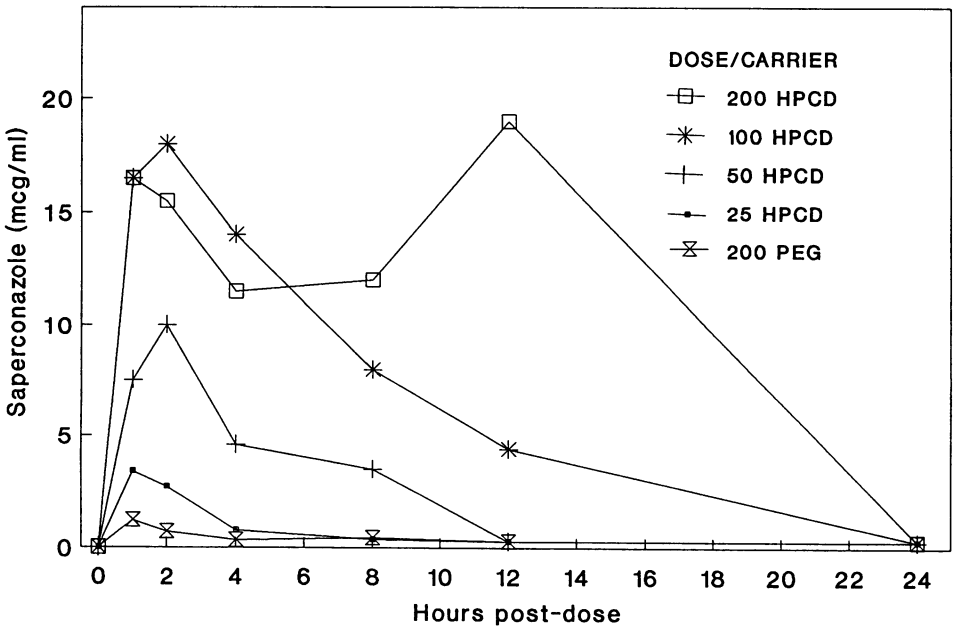

FIG. 3. Saperconazole concentrations over time derived from combined sera of two mice at each time point after a single dose. Drug was solubilized in either HPCD or PEG. Doses are in milligrams of saperconazole per kilogram.

TABLE 1. Pharmacokinetic results

Drug and carrier	Dose (mg/kg)	C_{max} (μg/ml)	$C_{max_{rel}}$[a]	AUC_{0-24} (μg · h/ml)	AUC_{rel}[a]
Itraconazole					
Cyclodextrin	200	24	24	363	121
	100	18	≥18	238	≥79
	50	13	≥13	168	≥56
	25	7	≥7	38	≥13
PEG	200	<1		3	
Saperconazole					
Cyclodextrin	200	19	19	274	21
	100	18	9	153	13
	50	10	10	50	10
	25	3	3	11	2
PEG	200	1		13	
	100	2		12	
	50	1		5	
	25	1		5	
Fluconazole					
Cyclodextrin	120	110	1	625	1
	60	43	1	225	1
	30	25	1	135	1
PEG	120	88		540	
	60	40		258	
	30	23		96	
Ketoconazole					
Cyclodextrin	40	22	2	75	2
PEG	40	11		48	
Miconazole					
Cyclodextrin	120	4	4	7	4
	60	1	1	4	1
	30	<1	1	<1	1
PEG	120	1		2	
	60	1		5	
	30	<1		<1	
SCH 42427					
Cyclodextrin	40	61	1	527	1
	20	21	1	186	1
	10	10	1	109	1
PEG	40	58		620	
	20	39		287	
	10	13		163	

[a] $C_{max_{rel}}$ and AUC_{rel} are relative to corresponding dose given with PEG or to highest dose given in PEG (results are expressed as equal to or greater than).

solution was mixed until it was homogeneous. The solution was cooled to 20 to 30°C. HPCD (60 g) was solubilized in 40 ml of purified water in a separate vessel and was then added to the drug solution, and the solution was mixed until it was homogeneous. The pH was adjusted to 1.9 to 2.1 with 10 N NaOH. Purified water was added to a final volume of 100 ml, and the solution was mixed until it was homogeneous. Drug dilutions were made by adding sterile water.

Drugs were also separately suspended in PEG (Sigma, St. Louis, Mo.) at room temperature.

Concentrations of itraconazole, saperconazole, ketoconazole, miconazole, SCH 42427, and fluconazole in serum were measured by using previously described bioassays (4, 7). The reproducibilities of these assays have been previously described in detail (7, 8). The lower limits of sensitivity of the assay, in micrograms per milliliters, were ≤0.08 for miconazole, 0.16 for ketoconazole, 0.16 to 0.31 for saperconazole, 0.31 for itraconazole, 1.0 to 2.0 for fluconazole, and 2.5 for SCH 42427. Preliminary experiments of the effects of excipients on bioassay zone sizes showed no difference between HPCD and PEG formulations for each of the drugs used in the study.

Ten-week-old female CD-1 mice (weight, 24 to 28 g; Charles River Laboratories, Inc., Portage, Mich.) were randomized by weight, and each was given an oral dose of the specified drug via gavage. At each time point in the study, two mice from each drug dosing group were bled and euthanized, and the sera were pooled for measurement of drug concentrations. Areas under the time-concentration curves from 0 to 24 h (AUC_{0-24}) were determined by the trapezoidal rule method as described previously (2).

Results of these studies (Fig. 2 and 3) revealed a marked elevation of concentrations of itraconazole and saperconazole in serum when they were solubilized in HPCD compared with those when they were solubilized in PEG. Furthermore, with HPCD there was a clear dose proportionality. Studies with 10 to 200 mg of itraconazole per kg of body weight in PEG revealed no dose proportionality (5). The AUC_{0-24} values with 10, 50, and 200 mg of itraconazole per kg in PEG were only slightly different.

Table 1 shows the peak concentrations (C_{max}) in serum and the AUC_{0-24} values for each of the drugs in the study, with comparisons of HPCD versus PEG as carriers.

The C_{max} and AUC for itraconazole (200 mg/kg) in HPCD were 24- and 121-fold higher, respectively, than a corresponding dose given in PEG. Even at a dose of 25 mg/kg, the C_{max} and AUC of itraconazole given in HPCD were 7- and 13-fold higher than those at a 200-mg/kg dose given in PEG. The C_{max} and AUC for saperconazole (200 mg/kg) in HPCD were 19- and 21-fold higher, respectively, than those for a corresponding dose given in PEG. Even at 25 mg/kg, the C_{max} of saperconazole in HPCD was threefold higher than that of a 200-mg/kg dose in PEG. The C_{max} and AUC_{0-24} for saperconazole (25 mg/kg) in PEG were essentially the same as (slightly higher than) those after a 50-mg/kg dose. Furthermore, whereas the AUC_{0-24} values for 100- and 200-mg/kg doses were greater than the AUC_{0-24} for either 25- or 50-mg/kg doses, the differences were nonlinear and the C_{max} for the 100-mg/kg dose was slightly higher than that for the 200-mg/kg dose. With saperconazole, it was notable that late (12 h) concentrations were particularly elevated at doses of 200 mg/kg in HPCD, possibly as a result of enterohepatic recirculation. Moderate enhancement of ketoconazole concentrations were noted, because both the C_{max} and AUC_{0-24} for ketoconazole (40 mg/kg) were twofold higher than those for a corresponding dose given in PEG.

No appreciable and consistent differences in C_{max} or AUC were seen with fluconazole, miconazole, or SCH 42427, regardless of the carrier. A comparative study with SCH 42427 (10 mg/kg) suspended in water revealed only slightly lower concentrations in serum in comparison with the concentrations of SCH 42427 suspended in PEG as the vehicle (data not shown). The highest concentrations, on a milligram-per-kilogram basis, of all drugs studied were achieved with SCH 42427, and the lowest concentrations were achieved with miconazole, which were barely detectable with the bioassay.

Because there were few observations during the elimination phase of each pharmacokinetic study, it is possible that the terminal triangle of each AUC was larger than it would have been with more observations. This could have distorted the stated AUCs and could have affected the ratio of AUC after drug administration with PEG to that after drug administration with cyclodextrin (AUC_{rel}). We therefore recalculated the AUCs for the period from 0 to 12 h. The AUC_{rel} for each drug was not substantially affected if these more restricted AUC data were used. The AUC_{rel} for the period from 0 to 12 h for the itraconazole and saperconazole doses studied ranged from ≥13 to 65 and 2 to 16, respectively, compared with ≥13 to 121 and 2 to 21, respectively, as given in the Table 1 for the AUC_{rel} for 0 to 24 h. The AUC_{rel} values for all fluconazole, ketoconazole, miconazole, and SCH 42427 doses studied were the same for the periods from 0 to 12 and 0 to 24 h.

As reviewed by Pitha et al. (6), a number of cyclodextrins have been developed for drug carrier systems through the substitution for hydroxyl groups, e.g., methylated, hydroxypropylated, or polymeric cyclodextrins. These have shown promise as carrier systems for a variety of drugs such as prostaglandins, barbiturates, steroids, and nonsteroidal antiinflammatory agents, among others. When they are taken orally, cyclodextrins are not absorbed appreciably and thus are not toxic (6).

Results of this study demonstrate that antifungal oral azoles, especially itraconazole and saperconazole, should be included as candidates for complexation with HPCD for enhancement of bioavailability. Further studies that examine the improved bioavailability of cyclodextrin-complexed itraconazole and saperconazole against systemic fungal infections would be of interest.

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REFERENCES

1. **Blum, R. A., D. T. D'Andrea, B. M. Florentino, J. H. Wilton, D. M. Hilligoss, M. J. Gardner, E. B. Henry, H. Goldstein, and J. J. Schentag.** 1991. Increased gastric pH and the bioavailability of fluconazole and ketoconazole. Ann. Intern. Med. **114**:754–757.
2. **Brass, C., J. N. Galgiani, T. F. Blaschke, R. Defelice, R. A. O'Reilly, and D. A. Stevens.** 1982. Disposition of ketoconazole, an oral antifungal, in humans. Antimicrob. Agents Chemother. **21**:151–158.
3. **Denning, D. W., R. M. Tucker, L. H. Hanson, and D. A. Stevens.** 1989. Treatment of invasive aspergillosis with itraconazole. Am. J. Med. **86**:791–800.
4. **Harvey, R. P., R. A. Isenberg, and D. A. Stevens.** 1980. Molecular modifications of imidazole compounds: studies of activity and synergy in vitro and of pharmacology and therapy of blastomycosis in a mouse model. Rev. Infect. Dis. **2**:77–86.
5. **McEwen, J. G., G. R. Peters, T. F. Blaschke, E. Brummer, A. M. Perlman, A. Restrepo, and D. A. Stevens.** 1985. Treatment of paracoccidioidomycosis with itraconazole in a murine model. J. Trop. Med. Hyg. **88**:295–299.
6. **Pitha, J., L. Szente, and J. Szejtli.** 1983. Molecular encapsulation of drugs by cyclodextrins and congeners. *In* S. D. Bruck (ed.), Controlled drug delivery. CRC Press, Inc., Boca Raton, Fla.
7. **Rex, J., L. H. Hanson, M. A. Amantea, D. A. Stevens, and J. E. Bennett.** 1991. Standardization of a fluconazole bioassay and correlation with results obtained by high-pressure liquid chromatography. Antimicrob. Agents Chemother. **35**:846–850.
8. **Sugar, A. M., S. G. Alsip, J. N. Galgiani, J. R. Graybill, W. E. Dismukes, G. A. Cloud, P. C. Craven, and D. A. Stevens.** 1987. Pharmacology and toxicity of high-dose ketoconazole. Antimicrob. Agents Chemother. **31**:1874–1878.
9. **Uekama, K., and O. Masaki.** 1987. Cyclodextrins in drug carrier systems. Crit. Rev. Ther. Drug Carrier Syst. **3**:1–40.
10. **Wishart, J. M.** 1987. The influence of food on the pharmacokinetics of itraconazole in patients with superficial fungal infection. J. Am. Acad. Dermatol. **17**:220–223.