

## Pharmacokinetics and Tissue Penetration of Fluconazole in Rabbits

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Received 27 September 1988/Accepted 24 January 1989

**Fluconazole is a new bis-triazole antifungal compound which has in vivo and in vitro activity against *Candida* spp. and *Cryptococcus neoformans* and excellent penetration into cerebrospinal fluid. However, little is known about the penetration of fluconazole into tissue sites other than cerebrospinal fluid. We therefore studied by high-pressure liquid chromatography the penetration of fluconazole into nine different tissue sites at times of peak and trough concentrations in plasma in rabbits. Fluconazole penetrated into all tissue sites. Tissue/plasma concentration ratios were greater at time of trough concentrations in plasma than at times of peak concentrations in plasma. The finding that fluconazole penetrated into target organs commonly infected by *Candida* spp. and *C. neoformans* further supports the therapeutic potential of fluconazole for disseminated candidiasis or cryptococcosis in immunocompromised hosts.**

Fluconazole is a new bis-triazole antifungal compound which has in vivo and in vitro activity against *Candida* spp. and *Cryptococcus neoformans* (10). Previous experimental and clinical studies demonstrated that fluconazole has excellent penetration into cerebrospinal fluid (1, 6, 8), and early clinical observations indicated that fluconazole is effective in the treatment of cryptococcal meningitis (3, 4). Little is known, however, about the penetration of fluconazole into tissue sites other than cerebrospinal fluid. A previous autoradiography study of mice injected with radiolabeled fluconazole demonstrated that activity appeared to be distributed throughout total body water (2). This method, however, was semiquantitative and did not distinguish between the parent compound and possible metabolites. Since other tissues clearly are important sites of invasive candidiasis and cryptococcosis in immunocompromised patients (5, 9, 11, 13), we studied the penetration of fluconazole into multiple tissues (lung, liver, spleen, kidney, choroid, vitreous humor, cerebrum, cerebellum, and skeletal muscle) of rabbits with a high-pressure liquid chromatography assay for fluconazole.

### MATERIALS AND METHODS

**Antifungal drug.** Fluconazole was kindly provided by Pfizer Central Research, Groton, Conn., as the parenteral formulation with a concentration of 1 mg of fluconazole per ml in 0.9% NaCl solution.

**Animals.** Adult female New Zealand White rabbits (Hazelton, Rockville, Md.) weighing between 2.5 and 3.5 kg were used in these studies. Rabbits were provided food and water ad lib, individually housed, and managed according to the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication no. 85-23, National Institutes of Health, Bethesda, Md.). Silastic central venous catheters were inserted under general anesthesia by a sterile surgical technique for repeated venous access as previously described (12). Hepatic and renal toxicities were monitored by biochemical profiles of blood urea nitrogen, alkaline phosphatase, and serum glutamic oxalacetic transaminase (Smith-Kline Bio-Science Laboratories) obtained on the first and last days of administration of fluconazole.

**Pharmacokinetics in plasma and tissue penetration.** Three groups of animals were studied. The first group of three rabbits received a single 5-min intravenous (i.v.) infusion of 25 mg of fluconazole per kg for the study of pharmacokinetics in plasma. Venous blood was sampled before dosing and 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10, 12, 24, 48, 72, and 96 h after i.v. infusion for the assessment of single-dose pharmacokinetics in plasma.

The second group consisted of three animals treated i.v. with 25 mg of fluconazole per kg per day for 14 days for the study of levels of fluconazole in tissue near times of peak concentrations in plasma. At 1 h after the final dose, animals were sacrificed with i.v. pentobarbital. Samples of plasma and sections of liver, spleen, lung, kidney, quadriceps, cerebrum, cerebellum, vitreous humor, and choroid were obtained immediately after euthanasia for the determination of concentrations of fluconazole in tissue near times of peak levels in plasma. Tissue was carefully dissected, with attention being directed toward avoiding contamination by blood. However, hemoglobin levels were not measured in tissue homogenates. Urine was drained from the urinary tract prior to dissection of the kidneys to avoid urinary contamination of renal tissue.

The third group of five rabbits received 25 mg of i.v. fluconazole per kg per day for 14 days for the study of fluconazole in tissue levels near times of trough concentrations in plasma. This group was sacrificed for the determination of concentrations in tissue when trough levels in plasma were reached, 24 h after the last dose. Plasma and tissue samples were frozen at  $-70^{\circ}\text{C}$  until assayed for fluconazole.

**Fluconazole assay.** Plasma samples were assayed for fluconazole by high pressure liquid chromatography (6). For the assay of concentrations of fluconazole in tissue, the entire tissue sample was weighed and homogenized with water (1:9 [wt/wt]). Tissue was homogenized in a Tissumizer (Tekmar, Cincinnati, Ohio) with a 10N head for 30 to 60 s. No signs of tissue heating developed with this method of homogenization. A sample of the resulting homogenate containing the equivalent of 100 mg of tissue (50 mg if 100 mg was not available) was assayed by the same procedure as that used for plasma. Control tissues mixed with known

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TABLE 1. Fluconazole concentrations in tissue and tissue/plasma concentration ratios near times of peak and trough concentrations in plasma

Tissue	Tissue concn ( $\mu\text{g/g}$ ) <sup>a</sup>		Tissue/plasma concn ratio <sup>b</sup>	
	Peak	Trough	Peak	Trough
Plasma	32.9 $\pm$ 13.1	5.8 $\pm$ 1.3	1.0	1.0
Liver	16.1 $\pm$ 1.6	14.9 $\pm$ 2.0	0.5	2.6
Spleen	23.4 $\pm$ 3.6	28.4 $\pm$ 7.4 <sup>c</sup>	0.7	4.9
Lung	26.3 $\pm$ 3.9	13.6 $\pm$ 3.0	0.8	2.3
Kidney	27.6 $\pm$ 2.7	18.7 $\pm$ 2.5 <sup>c</sup>	0.8	3.2
Quadriceps	21.0 $\pm$ 3.4	10.3 $\pm$ 1.8	0.6	1.8
Cerebrum	15.7 $\pm$ 0.9	9.4 $\pm$ 1.7 <sup>c</sup>	0.5	1.6
Cerebellum	21.6 $\pm$ 4.0	9.2 $\pm$ 2.7 <sup>c</sup>	0.7	1.6
Vitreous humor	15.7 $\pm$ 5.3	9.4 $\pm$ 1.9 <sup>c</sup>	0.5	1.6
Choroid	17.1 $\pm$ 5.6	9.8 $\pm$ 1.9 <sup>c</sup>	0.5	1.7

<sup>a</sup> Mean  $\pm$  standard error of the mean.

<sup>b</sup> Ratio of the means of concentrations in tissue and plasma for each tissue.

<sup>c</sup>  $P < 0.05$  for differences in concentrations between the kidney or spleen and the central nervous system (cerebrum, cerebellum, vitreous humor, and choroid).

amounts of fluconazole were used to establish standard curves. Preparations of control tissues were also examined to ensure that the extraction procedure did not produce peaks that would interfere with peaks of fluconazole or the internal standard.

**Data analysis.** For single-dose pharmacokinetics in plasma, the area under the plasma concentration-time curve was calculated by using the linear trapezoidal rule up to the final measured concentration and then extrapolating to infinity (7). The terminal half-life in the postdistributive phase was determined by regression analysis. The mean tissue/plasma concentration ratio for each organ was calculated as the ratio between the mean concentrations of fluconazole in tissue and in plasma. The significance of differences between concentrations of fluconazole in different tissues was determined by Student's *t* test (unpaired).

## RESULTS

**Single-dose pharmacokinetics in plasma.** The mean peak concentration of fluconazole in plasma in the first group was 29.3  $\pm$  1.36 (standard error of the mean)  $\mu\text{g/ml}$  following a 5-min i.v. infusion. The mean terminal half-life was 8.85  $\pm$  1.77 h, and the area under the concentration-time curve was 223  $\pm$  31.9  $\mu\text{g} \cdot \text{h/ml}$ . The volume of distribution was 853  $\pm$  29.9 ml/kg.

**Tissue penetration studies.** Fluconazole penetrated into all tissue sites at times of peak and trough concentrations in plasma (Table 1). Levels of fluconazole in tissue approximated those in plasma at the time of peak concentrations. The kidney had the highest mean tissue/plasma concentration ratio at the time of peak concentrations. Tissue/plasma concentration ratios at the time of trough concentrations were two to seven times the ratios obtained at the peak, indicating the persistence of fluconazole in tissue at low concentrations in plasma.

Concentrations of fluconazole were not significantly different among tissue sites at the time of peak concentrations in plasma. Concentrations of fluconazole at the time of trough concentrations in plasma, however, were significantly higher in the kidney and spleen than in the central nervous system (cerebrum, cerebellum, choroid, and vitreous humor). No nephrotoxicity or hepatotoxicity was detected.

## DISCUSSION

Fluconazole demonstrated extensive tissue penetration into multiple tissue sites throughout a 24-h dosing cycle. Since approximately 90% of fluconazole circulates in plasma as the unbound drug (2, 10), its apparent distribution into extracellular and intracellular free water would account for most of its large volume of distribution. Concentrations of fluconazole in tissue declined more slowly over time than did concentrations of fluconazole in plasma. Thus, tissue/plasma fluconazole ratios were higher at the time of trough concentrations in plasma than at the time of peak concentrations in plasma. These data suggest that the presence of fluconazole in tissues may continue to provide antifungal activity even at trough levels in plasma. Fluconazole penetrated into the commonly infected target tissues of candidiasis and cryptococcosis, suggesting that the compound may have considerable versatility in treating invasive mycoses at a variety of tissue sites.

A particularly important finding is the penetration of fluconazole into the parenchyma of the cerebrum and cerebellum throughout the 24-h infusion cycle. Fluconazole was demonstrated in a previous study to penetrate cerebrospinal fluid throughout a 24-h dosing cycle (1). Thus, fluconazole penetrates both cerebrospinal fluid and the brain parenchyma, suggesting a role for this agent against both meningeal and subcortical forms of cryptococcosis and candidiasis.

The penetration of fluconazole into the choroid and vitreous humor implies a potentially important role for fluconazole in the treatment of *Candida* endophthalmitis. High levels achieved in the liver and spleen suggest a possible role for fluconazole in the prevention or treatment of hepatosplenic candidiasis in cancer patients. The concentrations in the kidney may afford an alternative to amphotericin B and flucytosine in the management of *Candida* pyelonephritis, particularly when renal function is already compromised. The penetration of fluconazole into lung tissue suggests a role in the management of pulmonary cryptococcosis in acquired immunodeficiency syndrome patients and of hematogenous pulmonary candidiasis in cancer patients. Since fluconazole also penetrates skeletal muscle, it may be effective in the treatment of *Candida* myositis, which develops especially in granulocytopenic patients with disseminated candidiasis.

Little is known about the correlation between concentrations of antifungal compounds in tissue and their local antifungal activity in tissue. The penetration of an antifungal agent into a tissue suggests that the compound is potentially effective for the treatment of mycoses at that site. Conversely, the absence of penetration of an antifungal compound into a tissue suggests that the compound may not be effective for the treatment of infections at that site. Studies are in progress in our laboratory to investigate the correlation between local concentrations of fluconazole in tissue and its antifungal activity against experimental disseminated candidiasis in rabbits. Initial findings from these studies indicate that fluconazole has potent activity against disseminated candidiasis in multiple tissue sites.

That fluconazole penetrated into target organs commonly infected by *Candida* spp. and *C. neoformans* contributes further data to support the therapeutic potential of fluconazole for disseminated candidiasis or cryptococcosis in immunocompromised hosts.

## LITERATURE CITED

1. Arndt, C. A. S., T. J. Walsh, C. L. McCully, F. M. Balis, P. A. Pizzo, and D. G. Poplack. 1988. Fluconazole penetration into cerebrospinal fluid: implications for treating fungal infections of the central nervous system. *J. Infect. Dis.* **157**:178-180.
2. Brammer, K. W., and M. H. Tarbit. 1987. A review of the pharmacokinetics of fluconazole (UK-49,858) in laboratory animals and man, p. 141-149. *In* R. A. Fromtling (ed.), Recent trends in the discovery, development, and evaluation of antifungal agents. J. R. Prous Science Publishers, S.A., Barcelona, Spain.
3. Byrne, W. R., and C. P. Wajszczuk. 1988. Cryptococcal meningitis in the acquired immunodeficiency syndrome (AIDS): successful treatment with fluconazole after failure of amphotericin B. *Ann. Intern. Med.* **108**:384-385.
4. Dupont, B., and E. Drouhet. 1987. Cryptococcal meningitis and fluconazole. *Ann. Intern. Med.* **106**:778.
5. Edwards, J. E., R. I. Lehrer, E. R. Stiehm, T. J. Fischer, and L. S. Young. 1978. Severe *Candida* infections. Clinical perspective, immune defense mechanisms, and current concepts of therapy. *Ann. Intern. Med.* **89**:91-106.
6. Foulds, G., D. R. Brennan, C. Wajszczuk, A. Catanzaro, D. C. Carg, W. Knopf, M. Rinaldi, and D. J. Weidler. 1988. Fluconazole penetration into cerebrospinal fluid in humans. *J. Clin. Pharmacol.* **28**:363-366.
7. Gibaldi, M., and D. Perrier. 1982. *Pharmacokinetics*, 2nd ed., p. 445-449. Marcel Dekker, Inc., New York.
8. Perfect, J. R., and D. T. Durack. 1985. Penetration of imidazoles and triazoles into cerebrospinal fluid in rabbits. *J. Antimicrob. Chemother.* **16**:81-86.
9. Perfect, J. R., H. A. Gallis, and D. T. Durack. 1983. Cryptococemia. *Medicine (Baltimore)* **62**:98-109.
10. Saag, M., and W. E. Dismukes. 1988. Azole antifungal agents: emphasis on new triazoles. *Antimicrob. Agents Chemother.* **32**:1-8.
11. Thaler, M., B. Pastakia, T. H. Shawker, T. O'Leary, and P. A. Pizzo. 1988. Hepatic candidiasis in cancer patients: the evolving picture of the syndrome. *Ann. Intern. Med.* **108**:88-100.
12. Walsh, T. J., J. Bacher, and P. A. Pizzo. 1988. Chronic silastic central venous catheterization for induction, maintenance, and support of persistent granulocytopenia in rabbits. *Lab. Anim. Sci.* **38**:467-471.
13. Walsh, T. J., and P. A. Pizzo. 1988. Nosocomial fungal infections: a classification for hospital-acquired infections and mycoses arising from endogenous flora or reactivation. *Annu. Rev. Microbiol.* **42**:517-545.