ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1993, p. 903–904
0066-4804/93/040903-02$02.00/0

Vol. 37, No. 4

Uptake of Itraconazole by Alveolar Macrophages

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Received 20 December 1991/Accepted 25 January 1993

Itraconazole is a broad-spectrum potent triazole antifungal agent. Its efficacy in treatment cannot always be explained by body fluid drug levels. In this study, itraconazole was shown to accumulate into host cells. Its intracellular accumulation in cells is greater than that of the antibacterial agent clindamycin, which is known for intracellular localization, and the uptake process does not appear to be active. This ability to reach high concentrations intracellularly may be an important property for the in vivo efficacy of itraconazole.

Itraconazole is a promising new triazole antifungal agent which has been used successfully to treat a variety of deep-seated mycoses in animals and humans. In animal models, it has been as effective as fluconazole for treatment of cryptococcal meningitis despite undetectable drug levels in the cerebrospinal fluid (6); this finding contrasts with the high penetration of fluconazole into the subarachnoid space. These experiments showing success of itraconazole in treatment of central nervous system infections in animals (2, 6) were confirmed in human cases of cryptococcal meningitis (1, 7, 8). Possible explanations for the efficacy of itraconazole in the central nervous system include an effect of itraconazole on host cells by immune stimulation or intracellular accumulation of drug which is not detected by drug assays of biological fluids. We believe that part of the explanation resides in the latter hypothesis. Previously, we have shown that host cells can accumulate a large amount of itraconazole (5). The present study measured the uptake of itraconazole by alveolar macrophages and the factors which influence this interaction. Itraconazole was also compared with clindamycin, an antimicrobial agent known to accumulate inside macrophages.

Alveolar macrophages were obtained by lung lavage from 2- to 3-kg New Zealand White rabbits which had received 10^8 to 10^9 CFU of *Mycobacterium bovis* BCG intravenously 3 to 4 weeks before. In each assay, 10^7 cells per ml were used; each variable was tested in triplicate, and a mean value was recorded. A velocity gradient centrifugation assay was used to test for the partition of drug (4). Macrophages in Hanks' balanced salt solution containing tritium-labelled itraconazole supplied by Janssen Pharmaceutica, Beerse, Belgium, or tritium-labelled clindamycin supplied by Upjohn Co., Kalamazoo, Mich., were layered over a phthalate bed in microcentrifuge tubes. Cells and labelled drug were incubated in 5% CO_2 at 37°C for 10 min except as stated otherwise below. The cells were pelleted through phthalate at 15,000 × *g*. The supernatants and cell pellets were cut off at the bottom of the tube and placed in a 0.5% deoxycholate solution to lyse the cells. The supernatants and lysed cells were added to scintillation fluid for counting of disintegrations per minute. The compounds examined were [^{3}H]itraconazole (1 μg/ml), [^{3}H]clindamycin (10 μg/ml), [^{3}H]polyethylene glycol to measure extracellular volume, and ^3H_2O to measure total volume. Data were expressed as the ratios of intracellular drug concentration (C_{IN}) to extracellular drug concentration (C_{EX}).

Figure 1 shows the kinetics of intracellular uptake of itraconazole by alveolar macrophages. The uptake is both rapid and massive. Varying the pH of the medium from 4 to 9 had no significant effect on itraconazole uptake. There was also no significant change in uptake when the temperature was varied from 4 through 37°C. The effect of serum on itraconazole uptake, however, was significant. Itraconazole has previously been shown to be over 90% protein bound. With 5% serum in the medium, the C_{IN}/C_{EX} uptake ratio was approximately 18, compared with over 70 in cells placed in serum-free medium. When cells were placed in 100% serum, the C_{IN}/C_{EX} ratio dropped further to 3, but intracellular accumulation of drug did continue. Metabolic inhibitors such as carboxyl cyanide, *m*-chlorophenyl hydrazone, and potassium fluoride did not change uptake of itraconazole by viable macrophages.

Table 1 compares uptake of itraconazole and clindamycin by alveolar macrophages and erythrocytes. Itraconazole uptake by macrophages was eight times greater than clindamycin uptake, and unlike clindamycin, itraconazole did not require live cells for uptake because formalin-treated cells also accumulated itraconazole. Also, nonphagocytic cells such as erythrocytes trapped itraconazole. Therefore, the mechanism of itraconazole accumulation onto cells was not an active, energy-requiring process like clindamycin transport, which uses a nucleoside system (3).

When macrophages were examined for efflux of entrapped itraconazole and clindamycin into the medium, there was also a dramatic difference. Over a 60-min observation period, clindamycin was continuously released from the cells into the extracellular medium. On the other hand, less than 1% of itraconazole was released into the medium from intact, viable macrophages over this period.

Itraconazole actually appeared to facilitate its own binding to cells. When cells were preincubated with cold or unlabelled itraconazole or it was added during the incubation of [^{3}H]itraconazole with macrophages, there was a two- to fourfold increase in intracellular uptake of [^{3}H]itraconazole in the presence of cold drug over a 5- to 35-μg/ml range (Fig. 2).

For a quantitative measurement of biological activity of the intracellular itraconazole, 2.5×10^6 alveolar macrophages were incubated with 50 μg of itraconazole per ml for 2 h, washed extensively, and then lysed. The lysate was placed into agar wells with *Candida kefyr* added to the

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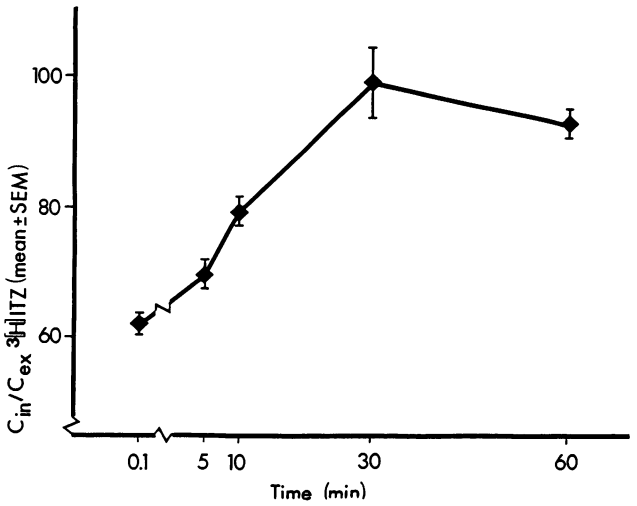

FIG. 1. Ratio of intracellular to extracellular concentration of 1 μg of itraconazole (ITZ) per ml incubated with alveolar macrophages over 60 min. SEM, standard error of the mean.

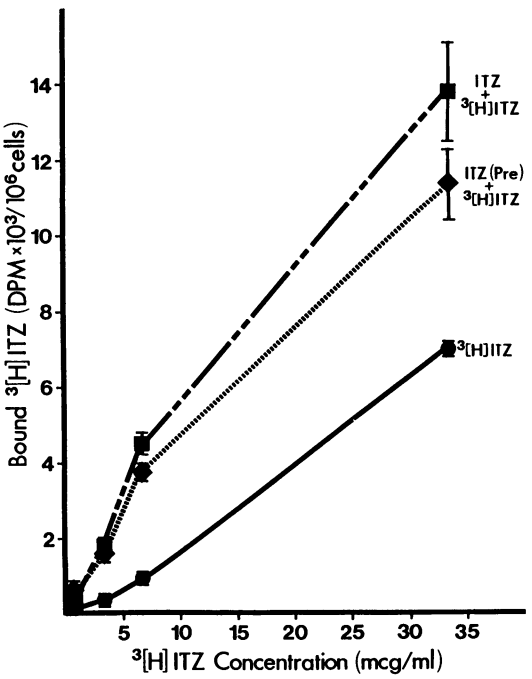

FIG. 2. Binding of labelled itraconazole (ITZ) to alveolar macrophages. Cells with labelled drug only were compared with those which were preincubated with 667 μg of cold itraconazole per ml for 1 h and those to which the cold drug was added at the same time as the [^{3}H]itraconazole. Data represent the mean ± the standard error of the mean.

medium. When compared with known drugs in control wells for inhibitory zone size, biologically active itraconazole released from lysed cells into extracellular medium was measured at 14.7 μg/ml of lysate.

Thus, the uptake of itraconazole by macrophages is both rapid and massive. Very little itraconazole is spontaneously released from these cells after uptake. Our findings suggest a primary physiochemical interaction, such as simple lipid solubility partitioning for this hydrophobic compound, rather than a specific receptor-mediated event or an active metabolic process such as that which occurs with clindamycin. Itraconazole also appears to facilitate its own accumulation within cells, since the presence of unlabelled drug significantly increases binding of the labelled compound. This facilitated accumulation may account for its C_{IN}/C_{EX} ratios being higher than those of other lipid-soluble antimicrobial agents. Also, this highly protein-bound molecule can still accumulate in cells despite competitive binding by extracellular serum proteins. Finally, the intracellular compound retains antifungal activity, and thus the physical interaction of itraconazole with host cells may be particularly important to its bioavailability at the site of infection. Pathogenic fungi may face a much higher concentration of itraconazole when they are taken up by host cells than is present in extracellular fluids such as serum and cerebrospinal fluid. This result may help explain its paradoxical effects on infections despite its low levels in biological fluids.

TABLE 1. Comparison of itraconazole uptake and clindamycin uptake by host cells in vitro

Drug	C_{IN}/C_{EX} (mean ± SEM)[a]		
	Alveolar macrophages		Erythrocytes
	Live	Formalin fixed	
[^{3}H]itraconazole	87.9 ± 5.3	55.4 ± 10.2	90.4 ± 10.1
[^{3}H]clindamycin	10.4 ± 1.7	2.5 ± 0.8	1.9 ± 0.1

[a] After 60 min of incubation with 1 μg of itraconazole per ml or 10 μg of clindamycin per ml.

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