Tissue Penetration of Antifungal Agents

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SUMMARY
Understanding the tissue penetration of systemically administered antifungal agents is critical for a proper appreciation of their antifungal efficacy in animals and humans. Both the time course of an antifungal drug and its absolute concentrations within tissues may differ significantly from those observed in the bloodstream. In addition, tissue concentrations must also be interpreted within the context of the pathogenesis of the various invasive fungal infections, which differ significantly. There are major technical obstacles to the estimation of concentrations of antifungal agents in various tissue subcompartments, yet these agents, even those within the same class, may exhibit markedly different tissue distributions. This review explores these issues and provides a summary of tissue concentrations of 11 currently licensed systemic antifungal agents. It also explores the therapeutic implications of their distribution at various sites of infection.

INTRODUCTION
Despite recent advances in antifungal chemotherapy, invasive fungal infections (IFI) remain a significant cause of morbidity and mortality (1). Candida species, Aspergillus fumigatus, and Cryptococcus neoformans are the most common pathogens (2). However, a wide range of other fungi, often with limited susceptibility to first-line antifungal agents, may also cause infection. Mortality from IFI remains high (e.g., that from aspergillosis is \( \approx 50\% \) [3, 4], and that from candidemia is 10 to 49\% [5, 6, 7]). An understanding of the pharmacological properties of any antifungal agent is crucial for optimizing patient outcomes for all these infections (8). This may be especially true for an increasingly recognized group of patients who have not previously been considered to be at high risk of IFI, such as critically ill patients and those with chronic obstructive pulmonary disease (COPD), who may demonstrate marked pharmacokinetic (PK) variability (9, 10).

Penetration into the site of infection to achieve microbe-eliminating concentrations is a key requirement for efficacy of all antimicrobial agents (11, 12, 13, 14, 15). The importance of tissue concentrations for the various classes of antibacterial agents has been reviewed extensively, but relatively less attention has been
This review examines the tissue penetration of 11 commonly used systemic antifungal agents (amphotericin B deoxycholate [AmBd], amphotericin B lipid complex [ABLC], liposomal amphotericin B [L-AMB], fluconazole, itraconazole, posaconazole, voriconazole, 5-fluorocytosine [5FC], anidulafungin, caspofungin, and micafungin) into the clinically relevant compartments for human infection and disease. All human data, ranging from case studies through autopsies to small clinical studies in volunteers or patients, were included. We also considered key laboratory animal data, where relevant, especially if the respective information for humans is absent. Because only free drug is considered to be biologically active (20, 21, 22), tissue and fluid concentrations are placed in context with the key physicochemical properties of each agent. The major organ systems covered include the lungs, liver, kidney, spleen, and heart. Attention has also been given to drug penetration into sanctuary sites (e.g., brain and eye), with the corresponding therapeutic implications. We have also reviewed the data for key interstitial fluids, including bronchial secretions, epithelial lining fluid (ELF), pleural fluid, pericardial fluid, synovial fluid, prostatic fluid, and cerebrospinal fluid (CSF), and placed these data in a clinical context (23).

**PENETRATION OF ANTIFUNGAL AGENTS INTO TISSUES: CONCEPTS, IMPORTANCE, AND CURRENT GAPS IN KNOWLEDGE**

**Importance of Tissue Concentrations for an Understanding of Antifungal Pharmacodynamics**

The potential relevance of the tissue concentrations of any anti-infective agent must be considered in context with the pathogenesis of the invading fungal organism (24). There must be colocalization of “drug and bug” within tissue beds and tissue subcompartments. Such considerations are relevant at the level of the organ and tissue subcompartments but may be elucidated further at the cellular and even molecular levels (25, 26, 27, 28, 29).

Most agents ultimately exert their effects on microorganisms residing within tissues. However, the distribution of agents from the bloodstream to various tissue subcompartments is often characterized by considerable variability, beyond that observed in plasma alone. Consequently, target site concentrations often differ markedly from those measured in plasma, especially in sanctuary sites such as the eye or central nervous system (CNS). Furthermore, there may be discordance in the shape of the concentration-time profiles for plasma and tissues. This phenomenon is called hysteresis (Fig. 1) and may explain persistent antifungal activity when plasma concentrations are low or undetectable (e.g., as seen with L-AMB [30], caspofungin [31], and itraconazole [76]). Conversely, suboptimal target site concentrations may well explain some cases of therapeutic failure (11, 13). In addition, as most fungal infections are extracellular, interstitial fluid may be the closest measurable compartment to the site of infection. However, the important compartment for prophylaxis may be different, which in turn is related to differences in pathogenesis and the stage of infection (Fig. 2A) (32, 33).

**Determinants of Distribution of Antifungal Agents into Tissues**

The principal chemical and pharmacokinetic properties influencing the tissue distribution of the 11 systemic antifungal agents in this review are summarized in Table 1. The four major classes of antifungal agents, i.e., the echinocandins, polyenes, pyrimidine analogues (5FC), and triazoles, are reviewed. These compounds are all distinct in terms of their chemical structure, molecular size, lipophilicity, and metabolism, and these differences have a major impact upon their pharmacokinetic and pharmacodynamic (PD) characteristics. Furthermore, there may be significant differences within a class. For example, the lipophilicities (expressed as log $D$ values in Table 1) of the four triazoles vary from 0.5 to 5.0, and plasma protein binding ranges from 12% to >99% (Table 1). These physicochemical properties determine the rate and extent of tissue penetration and bioavailability within a tissue, organ, or fluid (13, 34). Tissue and fluid concentrations for the three triazoles (fluconazole, voriconazole, and itraconazole), as multiples of those in blood or plasma, are shown in Fig. 3 to 5 to illustrate this.

In very general terms, small polar compounds with low plasma protein binding (e.g., fluconazole and 5FC) have volumes of dis-
A compound with an “intermediate” lipophilicity, volume of distribution, and plasma protein binding (e.g., voriconazole) is also predicted to distribute into aqueous sites but to attain relatively higher tissue concentrations than those of fluconazole or 5FC. In contrast, more lipophilic compounds (such as itraconazole and posaconazole) have much larger volumes of distribution (Table 1), tend to penetrate preferentially into tissues with high lipid content, and often exhibit tissue/plasma concentration ratios that exceed 1. Despite this, they may not necessarily penetrate well into sanctuary sites such as the brain, prostate, and eye. The polyenes (amphotericin B) and the echinocandins have variable tissue penetration but may also exhibit prolonged residence times.

A range of other factors may also have a significant impact upon tissue penetration, including (i) pharmacologic factors, e.g., route of drug administration, such as aerosol or parenteral therapy (35), or formulating drugs within lipids, e.g., amphotericin B colloidal dispersion (ABCD) and L-AMB (36), which may modify their distribution and alter their safety (37, 38) and potency (39); and
(ii) physiological factors, such as inflammation, which may increase tissue permeability, i.e., by disruption of normal physiological barriers such as the blood-brain barrier (29, 40); the underlying disease (41), which may result in a range of effects, including modification of plasma protein composition and hence drug binding (42, 43, 44); the recruitment of drug-containing phagocytic cells, i.e., the “dump truck phenomenon,” which may increase drug concentrations at the site of infection (12, 13, 32, 45, 46); drug export via pumps, e.g., for itraconazole and P-glycopro-

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mol wt* (particle size [μm])</th>
<th>Log D at pH 7.4</th>
<th>% Plasma protein binding</th>
<th>t1/2 (h)</th>
<th>AUC0–24 (mg · h/liter)</th>
<th>Vss b (liters/kg)</th>
<th>References</th>
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<tr>
<td><strong>Triazoles</strong></td>
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<td>Fluconazole</td>
<td>305</td>
<td>0.5</td>
<td>12</td>
<td>24–30</td>
<td>38</td>
<td>0.7</td>
<td>17, 18, 67</td>
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<td>Itraconazole</td>
<td>706</td>
<td>&gt;5</td>
<td>99.8</td>
<td>34</td>
<td>8.7–25</td>
<td>11</td>
<td>17, 226, 227</td>
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<td>Posaconazole</td>
<td>700</td>
<td>2.15</td>
<td>&gt;98</td>
<td>20–31</td>
<td>33–39</td>
<td>7–25</td>
<td>228, 229</td>
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<td>Voriconazole</td>
<td>349</td>
<td>1.8</td>
<td>58</td>
<td>6</td>
<td>13</td>
<td>4.6</td>
<td>84, 230</td>
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<tr>
<td>AmBd (conventional amphotericin B)*</td>
<td>924 (&lt;0.04)</td>
<td>−2.8</td>
<td>95–99</td>
<td>10–24</td>
<td>1–30</td>
<td>0.5–5</td>
<td>17, 144, 231–233</td>
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<td>ABLC (Abelcet)*</td>
<td>924 (1.6–11)</td>
<td>−2.8</td>
<td>95–99</td>
<td>24</td>
<td>9.5–14 ± 7</td>
<td>1.12–8.8</td>
<td>17, 144, 231, 232, 234</td>
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<td>L-AMB (Ambisome)*</td>
<td>924 (0.08)</td>
<td>−2.8</td>
<td>95–99</td>
<td>6–23</td>
<td>131 ± 126</td>
<td>0.11–0.7</td>
<td>17, 144, 233</td>
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<td>S-Fluorocytosine*</td>
<td>120</td>
<td>−2.34</td>
<td>5</td>
<td>3–5</td>
<td>576, 1289*</td>
<td>0.6–2.23</td>
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<td>Anidulafungin*</td>
<td>1,140</td>
<td>−3.32</td>
<td>84–99</td>
<td>26</td>
<td>110.3</td>
<td>0.8</td>
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<td>Caspofungin*</td>
<td>1,093</td>
<td>−3.88</td>
<td>97</td>
<td>9–11</td>
<td>57–96</td>
<td>0.15</td>
<td>235, 236</td>
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<td>Micafungin*</td>
<td>1,291</td>
<td>−1.62</td>
<td>&gt;99</td>
<td>15–17</td>
<td>29.6 ± 4.6</td>
<td>0.24–0.39</td>
<td>182, 235</td>
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*From reference 18.  
b Volume of distribution at steady state.  
* Dose-proportional pharmacokinetics.  
& Except in patients with renal impairment.  
* Concentration-dependent pharmacokinetics.  
f Data from oral solution and i.v. formulation in cyclodextrin.  
g Values for oral and i.v. formulations, respectively.

**FIG 3** Fluconazole tissue and fluid concentrations in humans as multiples of the maximal or simultaneously measured concentration in plasma (μg/ml) after systemic administration. Tissue multiples are from μg/g tissue values. Fluid multiples are from μg/ml concentrations. Numbers in parentheses indicate relevant references.

**FIG 4** Voriconazole tissue and fluid concentrations in humans as multiples of the maximal or simultaneously measured concentration in plasma (μg/ml) after systemic administration. Tissue multiples are from μg/g tissue values. Fluid multiples are from μg/ml concentrations. *, autopsy data; in these cases, the multiples are based on plasma Cmax values at the same dose in volunteers (188).
coma colleagues (51) highlighted the potential pitfalls in using drug leading matrix when used for this purpose. Mouton and colleagues (75); variable oral bioavailability, e.g., of itraconazole (47) and posaconazole (228); and interpatient variability in clearance, e.g., of voriconazole (48).

Limitations of Current Understanding and Approaches
Considering tissue concentrations in isolation is of limited value. A drug may be present at a site but at a concentration beneath the threshold required for activity, located in the wrong subcompartment, or not biologically available. Ideally, therefore, tissue concentrations should be analyzed with concomitant pharmacodynamic data. Examples of this problem include AmBd and itraconazole, which have low concentrations in the CSF yet are effective agents for treatment of cryptococcal meningitis (49, 50).

Tissue homogenates are frequently used to estimate tissue concentrations, but they are a relatively crude and potentially misleading matrix when used for this purpose. Mouton and colleagues (51) highlighted the potential pitfalls in using drug concentrations within whole-tissue homogenates for drawing conclusions related to the activity and efficacy of a drug, especially for extracellular pathogens. This may be a particular issue for amphotericin B (irrespective of formulation), where there is long-standing uncertainty related to the amount of biologically available drug in tissues. The potential reasons that tissue homogenates may provide inaccurate information regarding the “true” concentration at the site of infection include (i) discordance between intra- and extracellular drug concentrations versus where the pathogen is actually located, e.g., for posaconazole (33); (ii) multifocal versus diffuse disease, resulting in altered drug penetration at the site of infection compared with the normal contiguous tissue, e.g., pulmonary aspergilloma (257) or cerebral cryptococcoma (Fig. 6); (iii) the concentration of total versus biologically active drug, e.g., free amphotericin B versus drug that remains complexed to lipid (29, 52, 53, 54); and (iv) incomplete extraction of drug from tissue, e.g., for amphotericin B (29, 52, 53, 54).

Reporting tissue concentrations of anti-infective drugs in a clinically useful format is also problematic. One of the most common presentation methods is to use a ratio to plasma concentration, which may be flawed for a number of reasons. This ratio is dependent on both the denominator and the numerator, e.g., the bone tissue/plasma concentration ratio for ABLC in rabbits is 42, while the corresponding ratio for L-AMB is 0.66, suggesting that ABLC penetrates bone more effectively than L-AMB. However, the actual amphotericin concentrations achieved with the two lipid formulations in bone are similar (35.4 μg/g and 39.5 μg/g for ABLC and L-AMB, respectively) and, in both cases, superior to that achieved with AmBd (19). Comparison of concentrations taken at a single time point is also liable to induce errors because of hysteresis (Fig. 1), with a delay occurring as drug moves from the vascular to the tissue compartment (55). For this reason, it may be more useful to present the tissue area under the concentration-time curve (AUC) for comparison. There are few studies that do this for humans (56, 57, 58, 59, 60, 61, 62), and with one exception (59), all deal with pulmonary distribution.

Most of the antifungal agents considered in this review do exhibit hysteresis. This persistence of tissue concentrations may explain why, in specific situations, linking the tissue pharmacokinetic data with pharmacodynamic data produces a significantly more robust PK/PD model than using plasma PK data alone (31, 63). The technique of comodeling both PK and PD data may also produce a more insightful reflection of the impact of tissue concentration than the simplistic comparison of peak tissue concentration with the breakpoint MIC (64).

**FIG 5** Itraconazole tissue and fluid concentrations in humans as multiples of the maximal or simultaneously measured concentration in plasma (μg/ml) after systemic administration. Tissue multiples are from μg/g tissue values. Fluid multiples are from μg/ml concentrations.

**FIG 6** Cross section of the brain of a mouse with cryptococcal meningoencephalitis. The organism was stained with an antibody directed toward the cryptococcal capsule. The disease is multifocal. Attempts to use whole-brain homogenates to estimate drug concentrations at the site of infection may be misleading. (Reprinted from reference 263 by permission of the Infectious Diseases Society of America [taken by Julie Schwartz, Charles River Laboratories].)
The data were also potentially influenced by the underlying disease of the host. Consequently, we only used data where both plasma and tissue concentrations were reported within the same study (except for some postmortem studies in which tissue concentrations alone were reported).

Most human data are from healthy adult volunteers and/or a few patients, and their applicability to young children or neonates requires further study. Information is most comprehensive for the older triazoles (fluconazole and itraconazole), while both human and animal data for the newer agents (posaconazole and the three echinocandins) are more limited. Human data for AmBd (discovered in the 1950s) and 5FC (discovered in the 1970s) are also surprisingly sparse.

Despite the caveats discussed in the introduction, the published data are expressed as tissue or body fluid/plasma or blood concentration ratios. They are summarized in Fig. 7 as three differently colored ratio bands. The colors in the figure illustrate differing drug concentration ratio bands but do not imply differences in efficacy within various tissues or between drugs.

**Brain and Cerebrospinal Fluid**

The brain and CSF are sanctuary sites, as they are surrounded by lipid membranes with inward- and outward-facing transporters. Data from human studies suggest that fluconazole concentrations in CSF are dose dependent and vary between 50% and 100% of the concentration observed in the plasma (67, 68, 69, 70) (Fig. 3 and 7). Fluconazole is also readily detectable in human brain parenchyma. Studies with 18F-fluconazole in volunteers showed brain tissue concentrations that were similar to those in plasma, with some minor regional variation (71). However, in five surgical patients, fluconazole brain tissue/plasma concentration ratios of 0.7 to 2.4 were measured when the fluconazole plasma concentrations were at 90% of steady-state values (72).

**FIG 7** Concentrations in tissues and body fluids for each systemic antifungal agent relative to its concentration in plasma. X, human data; O, animal data. Colors illustrate differing ratios; multiple colors within a column give the range of published data. Red, from below level of detection to ≤0.5 times the plasma concentration; yellow, from >0.5 times to ≤5 times the plasma concentration; green, >5 times the plasma concentration; white, no data. ●, pleural fluid, buccal mucosa, or pancreatic pseudocyst; open diamond, based on autopsy data and human pharmacokinetics; O, wound fluid; ○, only detected in inflamed eyes; □, bronchial secretions; △, below level of detection in bronchial secretions; ◊, pulmonary lymph; ▼, bronchial biopsy specimen.
fluconazole in this model, even though fluconazole is readily detectable in rabbit CSF, with a CSF/plasma concentration ratio of 0.6 to 0.8 (77). Itraconazole also exhibits efficacy in human cryptococcosis, suggesting that it does penetrate the meninges and cerebral parenchyma and achieves the concentrations required for antifungal activity (50, 78).

Voriconazole has a lipophilicity that is intermediate between those of fluconazole and itraconazole (Table 1). Voriconazole penetrates human brain tissue (79, 80) and abscissae material (81), achieving peak concentrations similar to or even exceeding those seen in plasma (Fig. 4 and 7) (243). However, human CSF concentrations of voriconazole tend to be lower, with CSF/plasma concentration ratios of 0.22 to 1.0 (81, 82, 83). This is consistent with its intermediate plasma protein binding in humans of 58% (84). Voriconazole is the agent of choice for CNS aspergillosis (243). Posaconazole, which resembles itraconazole structurally but is less lipophilic (Table 1), also penetrates the CSF relatively poorly (85), with CSF/plasma concentration ratios of <0.009 (86). Its diffusion into the CSF may be increased by meningal inflammation. Thus, CSF concentrations in two patients with bacterial meningitis and cerebral fungal infection were 44% and 230%, respectively, of those in plasma (87). In mice infected with Cryptococcus gattii or Fonsecaea monophora, a bioassay revealed that brain tissue concentrations of posaconazole were approximately 53% of those in serum at daily doses of ≤20 mg/kg but increased to 70% to 80% at a daily dose of 40 mg/kg (88, 89).

Postmortem studies of humans show that amphotericin B is detectable, but only at low concentrations, in the brain tissue of patients receiving AmBd and L-AMB (52, 53, 90). Amphotericin B concentrations in the CSF are also low after administration of intravenous AmBd (91). Similar CSF and brain data for AmBd, L-AMB, and ABLC (i.e., CSF and tissue/plasma concentration ratios of <0.3) have been recorded for rabbits (92). To overcome these potential limitations, intraventricular instillation of AmBd via an Omnya reservoir has been used for severe cerebral infections (93, 94, 95).

The concentrations of 5FC in human CSF are similar to its corresponding serum concentrations (91, 96, 250), and a combination of 5FC with AmBd or L-AMB is a recognized first-line induction therapy for cryptococcal meningitis (97).

The three echinocandins, i.e., caspofungin, micafungin, and anidulafungin, are large, amphiphilic, cyclic peptides—properties that do not ordinarily favor penetration into the brain and CSF (98, 99). There are no human data for anidulafungin. However, its concentration in rabbit brains after multiple dosing is only about 10% of the maximum concentration of drug in serum (Cmax) (100, 101). Delivery of 14C-anidulafungin (as total drug-derived radioactivity) into the brains of rats is delayed compared to that into the blood and other tissues, and it is not detectable in brain tissue until 24 h after a single dose (102). In contrast, CSF concentrations are similar to those in the blood within 30 min of dosing (102). The administration of caspofungin to rodents results in brain tissue concentrations and exposures that are approximately 10% of those in plasma (103, 104). In a single patient with CNS coccidioidomycosis, CSF concentrations of caspofungin were undetectable, despite concentrations in plasma of 2.7 to 5.5 μg/ml (105). Similarly, the CSF/plasma concentration ratios of three patients receiving micafungin were low and variable, ranging from 0.002 to 0.54, while in the brain tissue of another patient, the tissue/plasma concentration ratio was only 0.17 (106, 107, 252). Micafungin penetration into rabbit brains is dose dependent, and significantly higher concentrations are measurable in the meninges than in either the cerebrum or cerebellum (108). However, the concentrations in these various subcompartments are also sufficient to achieve a significant anti-Candida effect. Animal models suggest equivalent efficacies between the echinocandins and amphotericin B formulations. The clinical value of the echinocandins for various fungal CNS infections remains to be established (18).

Eye

Endogenous fungal endophthalmitis, most commonly caused by Candida or Aspergillus spp., arises from hematogenous dissemination (109). A range of syndromes are seen, including chorioretinitis, vitritis, and pan-endophthalmitis. Successful therapy requires penetration of drug into the relevant subcompartment(s) of the eye, i.e., the choroid, retina, vitreous humor, and aqueous humor (16). For many antifungal agents, suboptimal penetration can mean that medical therapy alone is ineffective, and successful treatment may require vitrectomy and/or intracameral injection (Fig. 7).

Early human and animal data for azoles, polyenes, and 5FC have been well summarized elsewhere (16). Fluconazole (110, 111, 112), voriconazole (113, 114, 252), and 5FC (115, 116) are detectable in both the aqueous and vitreous humors of animal and/or human eyes, with and without endophthalmitis, at concentrations approximately 40% to 100% of those observed in serum. Although the use of 5FC is now uncommon, both triazoles are employed quite extensively for treating fungal ophthalmic infections in humans (109, 117). The visual adverse events experienced by some patients receiving systemic voriconazole are related to plasma exposure (258) but not yet to retinal concentrations per se. These adverse events, which have been ascribed to inhibition of the B wave of “ON” bipolar cells in the retina (118), do not appear to result in long-term adverse effects or toxicity (119).

Penetration of itraconazole into the eyes of rabbits after a single oral dose is minimal (120). No drug is detectable (using bioassay) in the aqueous or vitreous of uninflamed eyes, with only 0.3 μg/ml observed in the cornea, despite plasma concentrations of more than 10 times this value. With inflamed eyes, concentrations in the aqueous and vitreous are still 4- to 10-fold lower, respectively, than those in the plasma, while in the cornea they are low and unchanged relative to those in uninflamed eyes. Despite these results, itraconazole is as efficacious as ketoconazole and fluconazole against Candida albicans endophthalmitis in vivo when therapy is initiated within 24 h of infection (120). Similarly, a single patient with C. albicans endophthalmitis was treated successfully with 200 mg/day of itraconazole (capsules) and two vitrectomies (121). This was despite concentrations in the aqueous and vitreous humors that were undetectable and 0.02 μg/ml, respectively, while plasma concentrations were approximately 0.5 μg/ml. Heykants and colleagues (122) have also reported that itraconazole concentrations in human aqueous are usually only 1 to 2 ng/ml.

There are minimal data for posaconazole, but these suggest that it does penetrate into the inflamed eye. In a single patient with Fusarium solani keratitis and opthalmitis, receiving 200 mg orally (p.o.) four times daily plus topical instillation of the oral solution, the aqueous and vitreous/plasma concentration ratios were 0.6 and 0.21, respectively, and therapy was successful (244).

Two patients, with rhinofacial and orbital zygomycoses, each
Penetration of AmBd into both the aqueous and vitreous of the infected eye was higher in the patient with rhinocerebral disease and extensive retinal inflammation (fluid/plasma concentration ratio of 0.4) than in the second patient, who had minimal retinal inflammation (ratio of 0.06). Penetration of all formulations of amphothericin B into the eyes of rabbits is also enhanced by inflammation (124, 125, 249). Indeed, amphothericin B is not detected in noninflamed eyes, even after multiple dosing of AmBd, ABL, or L-AMB (16, 124, 125). Consequently, intracameral injection is the favored delivery route for these agents in patients with severe keratomycosis or endophthalmitis. For AmBd, this may lead to significant local toxicity, which is somewhat ameliorated by lipid formulations (109).

All three echinocandins also show limited penetration into the aqueous and vitreous humors of laboratory animals after systemic administration, with either undetectable or low concentrations relative to those in plasma (100, 109, 126, 127, 128). However, micafungin concentrations specifically in the retina and choroid of the eyes of rabbits range from 0.75 to 15.97 μg/ml and are comparable with the concentrations in plasma (129). As with amphothericin B, inflammation appears to improve the extent of echinocandin penetration (127). Potentially subtherapeutic vitreal penetration of caspofungin has been associated with treatment failure in *Candida albicans* endophthalmitis (130), and low concentrations of caspofungin were measured in the aqueous of one human endophthalmitis patient (113). Similarly, low micafungin concentrations in the aqueous and vitreous of a *C. albicans* endophthalmitis patient (0.001% of the simultaneous concentration in plasma) were associated with clinical failure (131), and the drug was also ineffective in a patient with endophthalmitis caused by *Candida tropicalis*, despite severe inflammation and a MIC of 0.03 μg/ml (132).

**Lung**

Pulmonary infection begins within the airspace (Fig. 2A). Therefore, for the agents used for prophylaxis or treatment of infection confined to the airspace, concentrations in epithelial lining fluid (ELF) and within pulmonary alveolar macrophages are of direct importance. The inhalation of aerosolized amphothericin B formulations is a potential option for prophylaxis (133, 134, 135). Antifungal drug concentrations within ELF after aerosol inhalation or systemic administration were recently reviewed (12). However, for treatment of established invasive infections, drug concentrations in the lung parenchyma may be more relevant (Fig. 2B and C). Drug concentrations may also be measurable in a number of other respiratory fluids, including bronchial secretions, sputum, pleural fluid, and pulmonary lymph (see below and Fig. 7).

Human studies suggest that 18F-fluconazole distributes rapidly into the lung tissue of volunteers, producing concentrations approximately double those in plasma (71). In 20 patients receiving a single 200-mg dose of fluconazole, the lung tissue/plasma concentration ratio varied from 1.1 to 1.6 (136). Similarly, the fluconazole ELF/plasma concentration ratio was 1.2 (137). Fluconazole also readily penetrates the extracellular space of the rat lung (fluid/plasma concentration ratio of 1.38), and this is unaffected by inflammation (138). Itraconazole exhibits ELF exposures that are one-third of the plasma AUC in human volunteers, while the AUC in alveolar cells is more than double that of the plasma (56). In postmortem samples from four hematology patients, the mean lung tissue/plasma concentration ratio of itraconazole was 7 (139), while Heykants and colleagues (73) reported concentrations 0.9 to 2.4 times higher than those in the plasma of four patients. However, itraconazole concentrations in bronchial alveolar lavage (BAL) fluid and airway tissue were 10-fold lower than those in plasma in a patient with allergic bronchopulmonary aspergillosis (ABPA) (140). Itraconazole has been used extensively to treat pulmonary fungal infections.

Postmortem studies show lung tissue homogenate concentrations for voriconazole that are comparable with the plasma concentrations (80, 141). In volunteers receiving an i.v. loading dose on day 1 and then 200 mg of voriconazole p.o. twice a day (b.i.d.), the ELF/plasma concentration ratio was 11 (142). However, in volunteers receiving the same i.v. loading dose on day 1, but followed by three doses of 4 mg/kg i.v. every 12 h (q12h), the ELF/plasma concentration ratio at steady state varied over 12 h from approximately 6 to 9, while for alveolar macrophages the ratio varied from approximately 3.8 to 6.5 (58). Posaconazole exhibits ELF concentrations in humans similar to those seen in the plasma, but the exposure in alveolar cells is over 30 times that in plasma in both volunteers (57) and lung transplant patients (143). It has been suggested that high intracellular posaconazole concentrations may explain its effectiveness for prophylaxis (Fig. 2A) (33). Mean lung tissue concentrations of posaconazole in rabbits have been reported to range from 0.3 μg/ml to 2.1 μg/ml after dosing at 2 to 6 mg/kg (145).

The administration of all formulations of amphothericin B results in quantifiable concentrations in the ELF in both rabbits and humans, but the plasma/ELF concentration ratios appear to differ between formulations and species. The precise state of the amphothericin in these studies is not clear (i.e., free, protein bound, or lipid associated). Furthermore, the biological relevance of the total concentrations associated with each formulation is also unclear. Human data for the various amphothericin formulations suggest that there may be some differences compared with rabbits (146, 147). Thus, intravenous ABL produces ELF amphothericin B concentrations that are approximately 4 times those produced after administration of L-AMB in humans (147). In 18 patients undergoing thoracotomy and resection for lung cancer, a single dose of 1.5 mg/kg i.v. of L-AMB resulted in hysteresis, such that tissue/plasma concentration ratios were 0.29 and 2.5 at 10 and 25 h postdose, respectively (248). In a postmortem study, lung tissue homogenate concentrations were found to be 3 times higher with a similar dose of ABL than with L-AMB (90). Similarly, ABLC concentrations in mouse lung homogenates exceeded those for equivalent doses of L-AMB (39). Pulmonary inflammation may increase amphothericin concentrations following administration of L-AMB (148). The amphothericin B formulations remain frontline agents for the therapy of pulmonary fungal infections.

There are no published data for echinocandin concentrations within human lung tissue. However, the concentrations of caspofungin in alveolar macrophages were >5 times the corresponding concentrations in plasma in a single patient (149). Both anidulafungin and micafungin also accumulated in the alveolar macrophages of volunteers, attaining concentrations approximately 14 and 4 times higher than those in plasma, respectively (58, 62). In 18 lung transplant patients receiving a single 150-mg i.v. micafungin dose, ELF/plasma and alveolar cell/plasma concentration ratios varied with time postdose. Mean ratios ranged from 0.1 to 1.53 at 3 h and from 1.1 to 6.2 at 24 h postdose (62). The vast majority of anidulafungin and micafungin found in the ELF is
present within macrophages rather than in the fluid itself (58, 61, 62). Caspofungin, micafungin, and anidulafungin exhibit lung tissue exposures in rodents that exceed those in plasma by 1.1-fold, 2.8-fold, and 10-fold, respectively (102, 103, 150).

**Pulmonary Lymph Fluid**

There are no human data for antifungal drug concentrations in pulmonary lymph, but Hoeprich and colleagues (151) examined the concentrations of 5FC and AmBd in sheep cannulated via the afferent duct of the right caudal mediastinal lymph node. All drugs tested (also including ketoconazole, the triazole Bay n733, and AmBd methyl ester [AME]) appeared promptly in the lymph after a single intravenous dose, with their concentrations subsequently decaying exponentially. In general, the concentrations of all five drugs in lymph slightly exceeded those in plasma measured shortly after the end of the 30-min infusion period (maximum ratio for lymph to plasma of 1.0 to 1.9), except for AME, where lymphatic concentrations were lower. Koizumi and colleagues (152) also examined AmBd concentrations in sheep lung and lymph after an i.v. infusion. The concentrations in the lymph were similar to (or slightly exceeded) those in the plasma, depending on the duration of the infusion. Given the range of lipophilicities and plasma protein binding of the above antifungal agents, these properties do not seem to have a significant impact on penetration into the lymphatic system, at least following intravenous administration.

**Pleural Fluid**

Data on antifungal drug pleural fluid concentrations are limited (Fig. 7). Voriconazole penetrates into the pleural fluid, producing trough concentrations in humans that are similar to paired plasma concentrations (153, 154). For AmBd, pleural fluid concentrations are approximately 50% of those in plasma (91, 247). However, pleural fluid amphotericin concentrations following the administration of L-AMB or ABCD are approximately 5% to 25% of their plasma exposures (60, 155). Penetration of the echinocandins into pleural fluid appears to be low. Thus, for anidulafungin in one patient with Candida empyema and for three micafungin patients, pleural fluid concentrations were less than 1% and 10%, respectively, of those measured in the plasma (107, 251).

**Bronchial Secretions**

Watkins and colleagues (140) demonstrated, for one patient, that itraconazole accumulates to approximately twice the plasma concentration in bronchial biopsy tissue and is also detectable (at only ng/ml concentrations) in BAL fluid and bronchial washings. However, no allowance was made for the significant dilution factor involved with their sampling methods. They concluded that itraconazole is present in “relatively high” concentrations in pulmonary fluids and tissues. In contrast, amphotericin B was detected, but only briefly postdose and at low concentrations, in the tracheal secretions of humans (91) and the tracheas of dogs following administration of AmBd (91), although penetration may be dose dependent (156). For 5FC, concentrations in dog bronchial secretions are approximately 75% of corresponding plasma concentrations (156).

**Saliva, Sputum, Buccal Mucosa, and Esophagus**

The attainment of effective antifungal drug concentrations within the saliva, sputum, and bronchial fluid is critical for therapy of oropharyngeal, esophageal, and bronchial infections. Fluconazole (67, 157, 158) and itraconazole (73, 159) have both been detected in the saliva and sputum of patients (Fig. 7). Consistent with their physicochemical properties (Table 1), the concentration ratios for fluconazole in saliva and sputum compared with serum are ~1, while for itraconazole they are generally much lower (73) and very variable (159). Itraconazole can also be detected in esophageal tissue, at 3 times the concentration in plasma (160), and in bronchial exudates (73). However, clinical data suggest that fluconazole is superior to itraconazole for treating oropharyngeal and esophageal candidiasis (161, 162). Voriconazole is present in the saliva of volunteers, and concentrations increase over time, using a standard dose. Thus, salivary exposure on day 1 is approximately 25% of that in plasma and increases to 88% of that in plasma with multiple dosing (163). Fluconazole and voriconazole show comparable efficacies in immunocompromised patients with esophageal candidiasis (164). While there are no published data for posaconazole concentrations in saliva, sputum, or mucosal and esophageal tissues, this drug is as effective as fluconazole in treating HIV patients with oropharyngeal candidiasis (165).

Buccal mucosal concentrations of amphotericin B increase in a dose-dependent manner in humans after L-AMB administration and attain concentrations approximately 7 to 43 times those in plasma (166). A wide range of amphotericin B concentrations were also detectable in esophageal autopsy samples from seven patients after AmBd administration (54). The concentrations of 5FC in human saliva are slightly lower than those in the plasma, but the 5FC concentrations measured in the bronchial secretions of dogs are comparable to serum concentrations (91).

There are no human or laboratory animal data giving the concentrations of caspofungin or micafungin at these sites. Anidulafungin is present in both the saliva and esophagus in rabbits with oropharyngeal and esophageal candidiasis, but only at concentrations between 1% and 33% of those in plasma (167). However, all three echinocandins show efficacy at the end of therapy equivalent to that of fluconazole after intravenous administration to patients with AIDS and oropharyngeal or esophageal candidiasis (168, 169, 170). There are no data to indicate whether any efficacy differences between fluconazole and the echinocandins seen on longer-term follow-up of these patients are related to residual tissue concentrations.

**Heart**

Fluconazole and voriconazole concentrations in human heart tissue are comparable to those in plasma, based on 18F-NMR studies in healthy volunteers and autopsy data, respectively (71, 80). The pericardial fluid/plasma concentration ratios of fluconazole in 20 patients ranged from 0.9 to 1.0 (136). Data from a single patient with disseminated aspergillosis also suggest that voriconazole diffuses into the pericardial fluid, at a concentration comparable to the plasma concentration (153). Autopsy data also indicate that myoccardial voriconazole concentrations are similar to those in other body organs, including the lung and kidney (80). In contrast, itraconazole exposure in the hearts of mice after a single 10-mg/kg i.v. dose is only 8% of that in plasma (171). However, in rats, at 1 h postdose, the concentration is 6 times the level in plasma, and both the absolute concentration and the plasma ratio increase further after 24 h (76). There are no published human heart tissue concentration data for itraconazole. Nevertheless, itraconazole can cause congestive heart failure (172) via negative
inotropic effects, although the precise mechanism is unknown (173).

Postmortem studies of patients following administration of AmBd or L-AMB show a wide range of concentrations (<0.1 to 9.1 μg/g) of amphotericin in heart tissue and myocardium (52, 90). In the hearts of dogs, the AmBd concentration after 14 days of dosing with 0.6 mg/kg/day is approximately 7 times the corresponding plasma value (37), while in rats given a single AmBd dose of 1.0 mg/kg, it is approximately 3 times higher (225).

As with fluconazole, the concentration of 18F-5FC in rat heart tissue is similar to that in blood (174).

Caspofungin is detectable in the rodent heart after a single dose, at a concentration approximately 20% of the peak plasma concentration, which then declines at a lower rate than in the plasma (103, 104). In contrast, anidulafungin exposure in the heart tissue of neonatal rats increases to approximately 1.3 times the concentration in plasma after a single dose and 1.8 times after multiple dosing (175).

### Liver

Given its major role in metabolism and clearance, many xenobiotics are likely to achieve higher concentrations in the liver than in the plasma. Twenty minutes after intravenous administration, the concentration of 18F-fluconazole in human livers is approximately 3 times the paired plasma concentration, while in rabbits it is twice that in the plasma (71). Itraconazole also accumulates in the liver (Fig. 7) (122), and it reached a concentration in one patient that was over three times that in plasma (73). However, in the livers of rats, itraconazole achieves concentrations that are approximately 13 times those in plasma 1 h after a single intravenous dose, and this increases further over 24 h (76). The plasma concentration declines 9-fold over this period, resulting in a tissue/plasma concentration ratio exceeding 150 at 24 h postdose (76).

In contrast, the nucleoside 18F-5FC, which is even more polar than fluconazole, attains concentrations in rat livers that are similar to those in plasma (174).

Hepatic concentrations of amphotericin are detectable from tissue obtained at postmortem (52, 54). There is a relationship between the plasma exposure of L-AMB and liver tissue concentrations of amphotericin B in human autopsy samples. After L-AMB dosing, the mean amphotericin B concentration that was achieved was 102 μg/g liver, but with substantial interpatient variability (90). Amphotericin B has a long residence time in hepatic tissue of mice. Concentrations (measured using bioassay) are detectable 14 days after dosing with L-AMB (38). However, Andes and colleagues (39) have shown that ABLC exhibits lower concentrations in mouse liver homogenates than equivalent doses of AmBd or L-AMB (at least following intraperitoneal [i.p.] administration).

The exposures of anidulafungin and caspofungin in the livers of rodents are raised approximately 10- and 16-fold, respectively, compared with plasma concentrations (102, 103). This is largely related to delayed clearance from the liver. However, micafungin appears to behave differently, with a lower peak concentration in the livers of rats and an AUC that is similar to that of the plasma (150). For caspofungin, specific hepatic transporters that mediate uptake into rat liver have been identified (26).

### Kidney

Approximately 80% of a fluconazole dose is eliminated as unchanged drug in the urine. Consequently, urinary concentrations are approximately 10 times those in human plasma (Fig. 7) (67). Fluconazole also readily penetrates kidney tissue, with peak tissue concentrations of 18F-fluconazole that are approximately 4 times the peak in human plasma (71). Similar to fluconazole, voriconazole is largely excreted via the urine (78%) and feces in humans, but mostly as metabolites, with less than 2% excreted as unchanged drug (84). Postmortem studies of eight patients showed that voriconazole was detectable in kidney tissue, at a mean concentration of 6.47 μg/g, but with significant interindividual variability (80). In contrast to fluconazole, itraconazole concentrations in urine are very low due to its negligible renal excretion (122). When administered intravenously to rats, itraconazole attains concentrations in kidney tissue of 5.5 μg/g after 1 h (3 times the plasma concentration) and 5.9 μg/g (31 times the plasma concentration) at 24 h postdose (76). However, a kidney tissue concentration of only 0.5 μg/g (1.5 times the plasma concentration) was recorded in a single patient (73).

The kidneys are a primary site of toxicity for all polyenes. Postmortem studies show that amphotericin B (from AmBd or L-AMB) is readily detectable in kidney tissue (52–54, 90). The renal concentration of amphotericin B in rat kidneys after AmBd administration is 10 times that in the serum, while the corresponding renal concentration after L-AMB administration is one-third that of AmBd and only 4 times the serum concentration (177). This is consistent with the reduction in amphotericin B-associated renal toxicity after its administration as L-AMB (or other lipid amphotericin formulations) rather than AmBd (178). The clearance of amphotericin B from the kidneys of rodents is prolonged, and the drug is detectable for at least 48 h after a single administration of AmBd (177) and at least 14 days after a prolonged course of L-AMB (38). In mouse kidney homogenates, concentrations of amphotericin B following administration of L-AMB or ABLC at a dose of 80 mg/kg i.p. are comparable to those observed with 20 mg/kg i.p. of AmBd (39).

Like fluconazole, 5FC is principally eliminated in the urine as unchanged drug (97%), and plasma clearance is closely related to creatinine clearance (91, 179). The concentration of 18F-5FC in rat kidneys is 3 times that in blood 2 h after dosing, with very high concentrations (60 times the plasma concentration) in the urine (174).

All three echinocandins readily penetrate into the kidney tissue of laboratory animals. After a single dose, 14C-anidulafungin exposure in rat kidney tissue is approximately 10 times that in plasma (102). In addition, anidulafungin exhibits an extended residence time in the kidney, with a terminal half-life that is twice that in plasma (102). Anidulafungin also accumulates in rabbit kidneys after multiple dosing (100). After a single dose administered to mice, caspofungin exhibits a longer mean residence time in the kidneys (31) and has a tissue/plasma concentration ratio over 24 h of approximately 7 (103). In contrast, micafungin concentrations in rat kidneys exceed those in plasma 5 min after dosing, by 1.6-fold, but then decline in parallel with plasma concentrations (150). All three echinocandins exhibit low concentrations (<2% of the dose) (104, 181, 182) of unchanged drug in human urine. There are reported cases of the efficacy of the echinocandins in patients with candiduria (183, 184), but this may reflect the attainment of high concentrations in renal parenchyma.

### Spleen

Fluconazole penetrates into the spleen in both humans and rabbits, although to different extents (71, 185, 213). Higher concen-
trations of $^{18}$F-fluconazole are seen in human spleens than in any other organ, with a tissue/blood concentration ratio of approximately 6 (Fig. 7). However, in rabbit spleens, concentrations are similar to those in the blood (71, 213) but slightly less than those in the plasma (185). Human data are limited for itraconazole, but splenic concentrations in two patients were 2- to 3-fold higher than the plasma concentrations (73, 186). However, a study in rats showed a progressive accumulation of drug in the spleen over the dosing interval, to approximately 10 times the plasma concentration (76). In contrast, mice receiving itraconazole at 20 mg/kg i.v. had splenic concentrations at 5 hours postdose that were 3 times those in the plasma, but they were similar to the plasma concentrations by 24 h (187). There are no laboratory animal data for voriconazole, but it is detectable in human splenic tissue at postmortem (80, 141). The splenic concentration (mean, 5.6 μg/g) is similar to the plasma steady-state concentrations in volunteers receiving 200 mg b.i.d. p.o. (188).

Human postmortem studies indicate that AmBd and L-AMB are detectable in the spleen at concentrations exceeding those of all other organs except the liver (52–54). Furthermore, tissue concentrations are dose dependent (53, 90). In dogs receiving 0.6 mg/kg/day of AmBd for 14 days, splenic concentrations are >160 times those in the plasma (37). After multiple dosing to mice, the splenic concentrations of amphotericin B derived from the three formulations are in the rank order ABLC > L-AMB > AmBd (35, 189).

There is little published information for 5FC, but concentrations of $^{18}$F-5FC in rat spleens are similar to those in blood (174).

There are no human data for the echinocandins, but all three agents are detectable in the spleens of laboratory animals (127). In the rat spleen, anidulafungin exposure is 10 times greater than that in plasma after a single dose of 5 mg/kg, and peak splenic concentrations exceed those measured in rabbits following multiple dosing at 10 mg/kg (100, 102). In contrast, the tissue/plasma concentration ratio of caspofungin is only ~1 after a single dose in mice (103) or rats (104). Miacafungin concentrations in rabbit spleens are also similar to those in plasma, even after multiple dosing (127).

Pancreas
Pancreatic antifungal drug concentrations are rarely reported for laboratory animals or humans. The most comprehensive data are for fluconazole, where 15 patients undergoing pancreatic surgery received a single fluconazole i.v. infusion of 400 mg (190). Pancreatic tissue concentrations increased for up to 2 h postdose, and the mean tissue/plasma concentration ratio at the time that tissue was sampled was approximately 1.0 (Fig. 7). Fluconazole penetration into pancreatic pseudocysts is slow, and concentrations attained in two cysts were lower than those in the plasma, at 0.4 and 0.8 times the plasma concentrations (190). Fluconazole concentrations in rat pancreatic tissue are similar to those in humans, with concentrations approximately 88% to 91% of those in plasma (190).

Penetration of AmBd into human pancreatic tissues has been demonstrated only in autopsy samples. Tissue concentrations are highly variable, ranging from <0.1 to 18.6 μg/g (52).

There are no data for caspofungin or anidulafungin, but a micafungin pancreatic pseudocyst fluid concentration of 0.38 μg/ml was recorded for a single patient 2 h after a prior dose (106).

Peritoneum
Intra-abdominal fungal infections are difficult to treat, particularly in patients requiring peritoneal dialysis (191). Fluconazole, 5FC, and amphotericin B are typically used as primary therapy, although limited experience in patients suggests that voriconazole, posaconazole, caspofungin, and micafungin could also be used for treating fungal peritoneal infection.

The polar agents fluconazole and 5FC achieve peritoneal concentrations after i.v. administration to uninfected laboratory animals of approximately 100% and 50% of those in serum, respectively (91, 192). Furthermore, in adults or children undergoing peritoneal dialysis, dialysate concentrations of fluconazole (following systemic administration) are similar to or exceed those in the plasma (193, 194). Limited clinical data suggest that the peritoneal concentrations of 5FC in humans are approximately 65% to 100% of those in serum (195, 196).

Five patients receiving voriconazole for peritonitis complicating peritoneal dialysis had concentrations in the peritoneal dialysate that were approximately 50% of those in the plasma after a single oral voriconazole dose (197).

The peritoneal concentrations of amphotericin B following AmBd administration are variable and less than 50% of serum concentrations (91, 195, 198) and, on occasion, are undetectable (196). Weiler and colleagues (199) have demonstrated that similar amphotericin B ascitic fluid concentrations are attained following administration of either L-AMB or ABLC for 7 to 13 days at 3 to 5 mg/kg/day.

A single patient receiving micafungin had a concentration in ascitic fluid of 1.02 μg/ml, giving an ascites/plasma concentration ratio of 0.15 (107).

Genital System
Fungal infections of the genital system, particularly vaginal candidiasis, are some of the most commonly experienced fungal infections of humans. Fluconazole is used extensively for treating urogenital infections caused by Candida spp. Consequently, there is a relative abundance of clinical data related to the concentrations of fluconazole within gynecological tissues and secretions (67, 200, 201), testicular (71) and prostatic (71, 202) tissues, and prostatic fluids (203) (Fig. 7). In the vagina and its secretions and in other gynecological tissues, the fluconazole tissue or secretion/plasma concentration ratio is at least 1. The tissue/plasma concentration ratio of fluconazole in the testicles of volunteers receiving $^{18}$F-fluconazole (71) is also ~1. However, in the prostate, which is a sanctuary site, the ratios range from 0.3 in prostatic hyperplasia patients (202) to 2.0 in volunteers (71). In the prostatic fluid of patients with AIDS and cryptococcal meningitis, the fluconazole fluid/plasma concentration ratio is 0.6 to 0.9 (203). The human data for itraconazole indicate that its concentrations in vaginal and other gynecological tissues and in cervical mucus are between 1.6 and 20 times those in plasma but that the vaginal fluid/plasma concentration ratio is <0.5 (73, 122). There are no published human or animal data for the other antifungal agents following systemic administration.

Bone
The concentration of $^{18}$F-fluconazole in bone is approximately 33% of the plasma concentration in humans and 100% in rabbits (Fig. 7) (71). After two i.v. doses, fluconazole is also detectable in the nucleus pulposus of the rabbit spine, but with a very wide
concentration range (0 to 63.5 μg/g) that is apparently unrelated to concentrations in plasma (204). In three patients, fluconazole synovial fluid concentrations were 0.88 to 1.0 times those in plasma (136, 205, 206). Similarly, the mean synovial fluid/plasma concentration ratio of fluconazole after 10 days of dosing to horses was 0.5 (207). Itraconazole may accumulate in bone, and the bone/plasma concentration ratio in a single patient was 4.7 (73). Voriconazole is detectable in human medullary and cortical bone, with especially high concentrations (approximately 5 times the plasma concentration) in the former (208). The concentration of voriconazole in synovial fluid from a single patient was approximately one-third the plasma concentration (208), while in horses, the mean voriconazole synovial fluid/plasma concentration ratio was 0.6 (209).

Amphotericin concentrations are high in the bone marrow of dogs and rabbits following administration of any of the currently available formulations (37, 210). Certainly the administration of amphotericin B in lipid formulations is considered an example of drug targeting, with particular respect to the kidneys and bone marrow (36). The lowest bone marrow concentrations are observed following administration of AmBd, but concentrations are still approximately 5 times those in plasma (37). In human synovial fluid, the measured AmBd fluid/plasma concentration ratio is approximately 0.4 (91), while in a single neonate with *C. albicans* osteoarthritis, the synovial fluid/plasma amphotericin concentration ratio of a random sample following 35 days of AmBd and 10 days of L-AMB was 1.4 (211).

There are limited data available on bone and synovial fluid concentrations of 5FC in humans and animals. Polak (91) reported bone and synovial fluid concentrations of 30% and 41% of those in plasma, respectively. However, in a premature infant with *Candida* arthritis, the synovial fluid concentration was approximately 83% of that in the plasma (212). In rats given 18F-5FC, bone and blood concentrations are comparable (174).

Anidulafungin concentrations in the bone of neonatal rats after a single dose are less than those in plasma, with a bone/plasma concentration ratio of 0.21 (175). No data are available for caspofungin or micafungin, although these drugs have been used to treat a few patients with bone/joint infections, in combination with AmBd or a triazole.

**Muscle**

The concentrations of 18F-fluconazole in human skeletal muscle are similar to those in the myocardium, both of which have a concentration ratio to blood of 1.8 (71). However, in rats and rabbits, the ratio is somewhat lower (0.58 to 0.74) (138, 185, 213). In contrast, itraconazole accumulates in skeletal muscle relative to plasma, attaining a muscle/tissue concentration ratio of 2.4 in a single patient (73) but one of over 7 in rats (76) (Fig. 7).

In human autopsy samples, skeletal muscle concentrations of amphotericin following the administration of AmBd ranged from 0 to 1.2 μg/g and were lower than those in any other tissue (54). In the rat, concentrations of amphotericin B in muscle (0.21 to 0.27 μg/g) were also lower than those in other tissues but were still approximately 10-fold higher than plasma concentrations following multiple dosages of AmBd (214). Simultaneously collected heart muscle tissue concentrations were approximately 20-fold higher than those in plasma. In autopsy samples from patients receiving L-AMB, the mean myocardial amphotericin concentration was 3.18 μg/g (90).

There are no human data for 5FC, but in rats receiving 18F-5FC, the skeletal muscle/blood concentration ratio is 1.1 (174). Human data are also lacking for the echinocandins. However, skeletal muscle concentrations of anidulafungin in rats are comparable to those in plasma (102), whereas for caspofungin, skeletal muscle concentrations in mice are less than 50% of those in plasma (103, 104).

**Skin and Nails**

The prolonged exposure of antifungal agents within the skin, nail, and nail bed is an important factor determining the outcome of treatment of dermatomycosis (215). Fluconazole concentrations within the dermis are similar to those in plasma (216, 217), but concentrations in the stratum corneum are up to 40 times those in plasma (217, 218) (Fig. 7). The clearance of fluconazole from the stratum corneum is also significantly slower than that from the plasma and other skin layers, with concentrations that decline 2 to 3 times more slowly than the plasma concentrations (215, 217, 218). Interestingly, once-weekly oral dosing of 150 mg for 2 weeks results in higher fluconazole concentrations in the stratum corneum relative to those in the epidermis/dermis, sweat, and serum than those obtained by daily dosing at 50 mg for 12 days (217). In fingernails, fluconazole concentrations are dose proportional and, at steady state, are approximately twice those in the plasma. Fluconazole is also detectable in nails up to 4 months after cessation of therapy (219). Slow clearance from both skin and nails is also seen for itraconazole. It binds tightly in the stratum corneum and does not readily distribute back to the plasma compartment (215, 220). The drug also accumulates in sebum. Consequently, those areas of skin with active sebaceous glands contain higher concentrations of itraconazole (e.g., the back, with twice the plasma concentration) than those that do not (e.g., the palm, with less than the plasma concentration) (122). Concentrations of itraconazole in blister fluid increase more slowly than those in the plasma, attaining a maximal concentration approximately 0.7 times that in the plasma (221). Itraconazole also has a very long residence time in nails after the cessation of therapy (122). Maximal concentrations of itraconazole in fingernails and toenails are 0.95 μg/g and 1.5 μg/g, respectively, 4 and 6 months after cessation of pulse therapy (222). The concentration of posaconazole within the human dermis is comparable to that in plasma (59). However, in toenails, its concentration is both dose and time dependent, attaining a maximum approximately 3 times greater than that in plasma after 24 weeks of therapy (223). There are no human data for voriconazole, but in guinea pigs, voriconazole skin concentrations are approximately twice those in blood, while in skin microdialysates, the voriconazole concentrations are only 50% of those in blood (224). Patients receiving voriconazole therapy have been shown to suffer from significant phototoxicity on exposure to sunlight, although a relationship to the voriconazole concentration or retinol levels in skin remains to be established (254). In a few patients, long-term voriconazole exposure may result in skin cancer (255, 256).

AmBd skin concentrations in rats receiving a single intravenous dose of 1.0 mg/kg are approximately 30% to 50% of those in plasma and decrease with time in parallel with the plasma concentrations (225).

Laboratory animal studies show that clearance of anidulafungin and caspofungin from rat skin is delayed compared to that from plasma, but these drugs never attain the peak concentrations mea-
sured in plasma (102, 104). After a single i.v. dose of anidulafungin, peak skin concentrations are approximately 80% of those in plasma, while for caspofungin, skin concentrations in rats peak at some 2 h postdose, but with a skin/plasma concentration ratio of only 0.3. However, caspofungin clearance from the skin is such that by 288 h postdose, residual skin concentrations, while only 15% of their peak, are 4 times those remaining in the plasma (104).

**UNDERSTANDING TISSUE CONCENTRATIONS FOR OPTIMAL USE OF EXISTING AGENTS AND DEVELOPMENT OF NEWER ANTIFUNGAL AGENTS**

### Current State of the Art

This review provides a summary of tissue concentration data for key antifungal drugs in humans and some animals. While there is a sizeable body of literature on this topic, many of the data are of variable quality, and the implications for the clinical care of patients with invasive fungal infections are frequently unclear. In addition, the human data are almost exclusively from adults, meaning that the implications for young children and neonates remain uncertain (65). The interpretation of many studies is further compounded by a multitude of different methodological approaches. Nevertheless, the following general conclusions seem reasonable.

1. First, small polar agents with low protein binding (e.g., fluconazole and 5FC) distribute more evenly and into a wider range of tissues than the larger, more lipophilic (itraconazole) or amphipathic (e.g., amphoterican B and echinocandins) agents.

2. Second, the more lipophilic or amphipathic agents may have longer residence times within tissues and may also accumulate to concentrations that exceed those in the plasma.

3. Third, agents with relatively low molecular weights, such as fluconazole, 5FC, and voriconazole, penetrate more readily into tissue beds.

4. Fourth, the formulation may have a significant impact on serum and tissue pharmacokinetics, although the pharmacodynamic implications of these differences frequently remain unclear.

5. Fifth, the measurable concentration of a drug within a tissue may not necessarily be an indication of its biological activity in that compartment.

6. Sixth, within a single drug class and with apparently closely related structures, there may be marked differences in tissue distribution (e.g., the triazoles).

7. Finally, a degree of caution is always advisable in extrapolating data from laboratory animals to humans.

### Beyond State of the Art

A detailed understanding of tissue concentrations is an important component of drug development (13). In this regard, the following are worthy of consideration.

1. First, comodeling both PK and PD data (if possible) provides key insights into the importance of tissue concentrations (64).

2. Second, single point estimates of tissue concentrations are of relatively limited value. Estimating concentration-time profiles (and thereby calculating the AUC in tissues) is possible using population pharmacokinetic modeling techniques. Relatively few studies have done this for humans to date (56–62), and all but one (59) deal with pulmonary distribution.

3. Third, designing antifungal regimens that optimize exposure at the site of infection rather than plasma exposure requires further consideration and study but may be pivotal in the design of optimum regimens for new antifungal agents (259).

4. Fourth, as has long been understood (51), tissue homogenates are not the ideal matrix for estimating tissue concentrations. Non-invasive methods such as magnetic resonance spectroscopy with spectroscopic imaging (e.g., 18F-5FC [260]) or positron emission tomography (e.g., 18F-fluconazole [71, 213]) can be used in laboratory animals or humans. Direct molecular analysis of whole-body animal tissue or isolated organs by matrix-assisted laser desorption ionization (MALDI) mass spectroscopy also represents a promising approach, without the requirement for radiolabeled drug (261).

### ACKNOWLEDGMENTS

Timothy Felton is an MRC Clinical Training Fellow supported by the North West England Medical Research Council Fellowship Scheme in Clinical Pharmacology and Therapeutics, which is funded by the Medical Research Council (grant G1000417/94909), ICON, GlaxoSmithKline, AstraZeneca, and the Medical Evaluation Unit.

Peter F. Troke was previously an employee of and a consultant to Pfizer and owns shares in Pfizer and other pharmaceutical companies. William W. Hope has acted as a consultant and received research support from Gilead Sciences, Pfizer Inc., Astellas, Merck, and F2G.

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