ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1989, p. 362–368
0066-4804/89/030362-07$02.00/0

Vol. 33, No. 3

Tissue Concentrations and Bioactivity of Amphotericin B in Cancer Patients Treated with Amphotericin B-Deoxycholate

N. COLLETTE, P. VAN DER AUWERA, A. PASCUAL LOPEZ,† C. HEYMANS, AND F. MEUNIER*

Service de Médecine Interne et Laboratoire d'Investigation Clinique Henri Tagnon, Institut Jules Bordet, Centre des Tumeurs de l'Université Libre de Bruxelles, rue Héger Bordet 1, 1000 Brussels, Belgium

Received 18 July 1988/Accepted 14 December 1988

We have studied amphotericin B concentrations in tissues of 13 cancer patients who died after having received 75 to 1,110 mg (total dose) of amphotericin B-deoxycholate for suspected or proven disseminated fungal infection. Amphotericin B concentrations were measured by high-pressure liquid chromatography (HPLC) and by bioassay, the latter being done on tissue homogenates as well as on tissue methanolic extracts. The fungistatic and fungicidal titers of the tissue homogenates were also tested against three strains of *Candida albicans* and one strain of *Aspergillus fumigatus*. Tissue concentrations of amphotericin B measured by HPLC varied with the tested tissues as well as with the total dose of amphotericin B-deoxycholate administered and ranged from 0.4 to 147.1 μg/g. A mean of 38.3% (range, 23.0 to 51.3%) of the total dose was recovered by HPLC from all of the tested organs. Bioassay of tissue methanolic extracts reached 58 to 81% of the concentration measured by HPLC, whereas only 15 to 41% was recovered from the homogenates. Overall, 27.5% of the total dose was recovered from the liver, 5.2% was recovered from the spleen, 3.2% was recovered from the lungs, and 1.5% was recovered from the kidneys. The median concentration in bile was 7.3 μg/ml, suggesting that biliary excretion could contribute to amphotericin B elimination to an estimated range of 0.8 to 14.6% of the daily dose. Fungicidal titers were seldom measured in tissues, but fungistatic titers were observed and were linearly correlated with amphotericin B concentration measured by HPLC. In conclusion, only a small proportion of the amphotericin B administered as amphotericin B-deoxycholate to patients seems diffusible and bioactive.

Amphotericin B remains the therapy of choice for invasive fungal infections in immunocompromised patients (2, 8), but treatment failures are still numerous, especially in patients with severe and persisting neutropenia (9, 16). Despite the widespread use of amphotericin B during the last 30 years, the pharmacokinetic profile of this agent in humans remains unclear. Concentrations of amphotericin B in serum measured after the administration of conventional doses (0.4 to 1 mg/kg per day) range from 0.5 to 2 μg/ml and usually exceed the MIC for most pathogenic fungal strains. However, the distribution phase and the elimination of the drug from the bloodstream seem to be rapid and followed by a prolonged beta phase of approximately 15 days (1). Taking into account the rapid dissemination of fungal infections in immunocompromised patients, the role of tissue concentration of amphotericin B for therapeutic efficacy is controversial, as suggested by a previous study performed on eight patients (6). In that study, the total recovery of amphotericin B calculated in only six patients ranged from 15.8 to 50.8% of the given dose; however, assays were performed on a limited number of different organs. Those authors suggested, based on a study in dogs (7), that bile could be a major route of excretion, although no data from humans were provided. They also suggested that amphotericin B could be stored in the tissues in a bioinactive form, although no metabolism could be substantiated. The measure of the bioactivity of amphotericin B was performed with a strain of a nonpathogenic mold. We further investigated in 13 cancer patients the tissue concentrations of amphotericin B determined by high-pressure liquid chromatography (HPLC) as well as the amount of bioactive drug. In addition to performing the bioassay previously reported (6), we assessed the bioactivity of amphotericin B in tissues by measuring fungistatic and fungicidal titers against various strains of pathogenic fungi.

MATERIALS AND METHODS

Patients. This study included 13 patients, 11 with hematological malignancies and 2 with solid tumors, who had received amphotericin B-deoxycholate (E. R. Squibb & Sons, Princeton, N.J.) by intravenous infusion and died from their cancer or its complications. Specimens of the liver, spleen, lungs, kidneys, and other tissues, when possible, were sampled and stored at −20°C until analyzed. The weights of each organ and sample were recorded. Histological examination and cultures were performed on all tissue samples. In addition, tissue specimens obtained at autopsy from three cancer patients who had never received amphotericin B-deoxycholate were used as controls.

Preparation and analysis of tissue samples for HPLC. (i) Homogenization. Tissue (0.5 g, wet weight) was homogenized in 1 ml of distilled water in a Potter grinder (Thomas, Philadelphia, Pa.) in melting ice. The homogenate was further diluted with 1 ml of distilled water.

(ii) Extraction method. Three volumes (0.9 ml) of methanol were added to 1 volume (0.3 ml) of tissue homogenate. The mixture was then centrifuged at 2,650 × *g* for 10 min. The clear supernatant was collected and assayed by HPLC and bioassay.

(iii) Chromatographic conditions. Amphotericin B was assayed as previously reported (5). Amphotericin B stock solutions (5,000 μg/ml) were prepared monthly from the commercially available preparation of amphotericin B-deoxycholate (Fungizone) and stored at 4°C. A complete standard curve was realized monthly in a pool of normal

* Corresponding author.
† Present address: Servicio de Hematologia y Oncologia, Hospital Clinico Universitario, Valencia, Spain.

TABLE 1. Characteristics of the patients

Patient no.	Age (yr)/sex[a]	Wt (kg)	Total dose of amphotericin B (mg)	No. of infusions	Time between last infusion and death (h)
1	39/F	85	75	2	16
2	49/M	75	90	1	27
3	63/F	54	144	4	2
4	52/F	58	175	3	72
5	60/M	67	229.5	6	7
6	39/M	83	250	3	29
7	55/M	58	300	6	10
8	52/M	75	300	6	44
9	75/M	65	400	10	17
10	66/M	80	440	9	18
11	66/M	67	500	5	72
12	77/M	83	1,050	21	69
13	32/F	49	1,110	31	44

[a] F, Female; M, male.

human serum by using the following concentrations of amphotericin B: 10, 5, 4, 3, 2, 1, 0.75, 0.5, 0.25, and 0.1 μg/ml. Assays were performed in duplicate. A working solution was made daily to prepare the external standards (2, 5, and 10 μg/ml) in a pool of normal human serum. These standards were assayed in duplicate every 10 tissue samples. The sensitivity of the technique was defined as the lowest concentration detected in spiked drug-free tissue specimens. The reproducibility was studied by using drug-free liver and spleen homogenates spiked with amphotericin B at the following concentrations: 0.5, 5, and 10 μg/ml. The intra-assay reproducibility was studied by repeating the extraction procedure 10 times. The between-assay reproducibility was studied by repeating the procedure over 9 days.

Efficiency of recovery by methanol extraction was studied by preparing homogenates of different tissues from a control patient. Standard solutions of amphotericin B were added before homogenization to obtain final concentrations of 0.5, 5, and 10 μg/ml of homogenate. Four homogenates were prepared for each concentration, extracted as previously described, and tested in duplicate in HPLC. Recovery was calculated as (amount of drug measured/amount added) × 100. No correction for blood contamination was done. To detect whether amphotericin B was metabolized, the difference between each sample and corresponding control retention times was calculated. For each organ, the mean and standard deviations of these differences were calculated.

Bioactivity of amphotericin B. (i) Bioassay. Amphotericin B concentrations were measured in tissue homogenates and in methanolic extracts from nine patients (patients 1, 2, 4, 5, 6, 9, 10, 11, and 12; Table 1) by radial diffusion in agar using a strain of *Paecilomyces variotii* (3). The spore-containing medium was distributed into 223- by 223- by 18-mm diffusion plates (Nunc, Roskilde, Denmark). In each plate, 36 wells were punched with a 3-mm puncher, and wells were filled with 30 μl of standards or tissue samples, tested in triplicate. Standards were prepared in a pool of sterile serum from healthy donors and spiked with known amounts of amphotericin B-deoxycholate. When tissue homogenates were tested, the standard curve (0 to 3.2 μg/ml by twofold dilutions) was prepared in serum. When methanolic extracts of tissue homogenates were tested, the standard curve (0 to 12.8 μg/ml) was prepared in serum extracted with an identical procedure.

Neither normal serum nor methanolic extract without amphotericin B produced an inhibition zone. Reproducibility was studied in spiked homogenates and corresponding extracts of liver and spleen at concentrations of 1 and 2.5 μg of amphotericin B per ml. Intra-assay reproducibility was studied by repeating the bioassay six times for the same sample. Between-assay reproducibility was studied by repeating the procedure over 7 days.

(ii) Fungistatic and fungicidal activities. Fungistatic and fungicidal activities were measured as previously described (15) by using tissue homogenates against clinical isolates: three fungemic strains of *Candida albicans* and one strain of *Aspergillus fumigatus*. Each tissue homogenate (50 μl) was serially diluted in duplicate in microdilution plates (final volume, 100 μl) by using an initial inoculum of 1.5×10^5 CFU or spores per ml. The tissue fungistatic titer (TFS) was defined as the highest dilution of the homogenate inhibiting visible growth of the tested strain.

After incubation, 20 μl of each well without visible growth was spread on a Sabouraud agar plate and subcultured for 24 h at 37°C. The tissue fungicidal titer (TFC) was defined as the maximal dilution of the homogenate which showed a 99% reduction of the initial inoculum (19). Results were expressed as reciprocal titers.

The in vitro susceptibility of the three strains of *C. albicans* and one strain of *A. fumigatus* was determined by using the method of Shadomy et al. (21). Amphotericin B (50 μl) was serially diluted in microdilution plates from an initial concentration of 100 μg/ml of yeast nitrogen base (YNB). The growth of the test organisms was controlled in drug-free wells. The MIC was defined as the minimal concentration of amphotericin B inhibiting visible growth of the tested strains. The minimal fungicidal concentration was defined as the minimal concentration of the drug producing a 99.9% reduction of the initial inoculum. Two media were tested: YNB and YNB supplemented with 25% pooled human serum.

Correlation between observed and expected tissue TFSs. Expected TFSs were calculated by dividing the concentration of amphotericin B (as measured by HPLC) by the MIC for each strain of *C. albicans*. The linear correlation between these titers and the corresponding observed titers was calculated, and the Pearson coefficient of correlation was determined for each strain.

RESULTS

Patients. The characteristics of the patients included in this study (four women and nine men) are as described in Table 1. The total doses of amphotericin B-deoxycholate administered ranged from 75 to 1,110 mg with a mean of 373.6 mg and a median of 300 mg (number of infusions per patient, 1 to 31). The regimens for intravenous infusions of amphotericin B-deoxycholate were either 0.6 mg/kg each day or 1.2 mg/kg every other day. The median duration between the last infusion and autopsy was 27 h (range, 2 to 72 h). Eleven patients had hematological malignancies, and two had solid tumors (patients 3 and 9). Fungemia was documented in four patients (patients 1, 5, 6, and 9) and was caused by *C. albicans* in patients 1 and 5, *Candida pseudotropicalis* in patient 6, and *Candida tropicalis* in patient 9. The autopsy results showed that patient 1 had disseminated candidiasis (*C. albicans*), patients 2, 3, 10, and 12 had pulmonary aspergillosis (*A. fumigatus*), patient 5 had pulmonary candidiasis (*C. albicans*), and patient 6 had esophagitis (*C. pseudotropicalis*). In six other patients (patients 4, 7, 8, 9, 11, and 13), no histological evidence of fungal infection

TABLE 2. Tissue concentrations of amphotericin B measured by HPLC

Patient no.	Amphotericin B concn (μg/g) in:						
	Liver	Spleen	Lungs[a]	Kidneys[a]	Pancreas	Brain	Heart
1	10.1[b]	12.9[b]	1.6[b]	3.3[b]	<0.1	0.2	<0.1
2	11.9	12.8	0.4[c]	2.5	<0.1	0.2	0.6
3	31.0	NT[d]	6.2[c]	11.0	3.9	0.3	2.7
4	35.3	23.6	4.1	7.9	8.1	NT	2.1
5	34.0	19.4	0.4[b]	4.0	18.6	NT	0.2
6	22.5	28.2	2.5	8.5	5.6	NT	<0.1
7	32.3	NT	8.1	7.3	5.7	0.5	1.1
8	24.2	4.3	2.1	4.8	5	0.3	0.4
9	45.6	50.2	7.7	8.7	0.8	0.7	0.6
10	35.6	8.6	5.9[c]	9.8	NT	NT	<0.1
11	62.3	32.7	4.5	8.7	13.1	0.7	NT
12	147.1	68.1	12.4[c]	30.4	NT	0.5	2.2
13	105.2	55.0	12.9	28.3	NT	5.8	5.7

[a] Mean value between right and left organ.
[b] Histologically proven candidiasis.
[c] Histologically proven aspergillosis.
[d] NT, Not tested.

was demonstrated at autopsy. In seven patients (patients 3, 4, 6, 9, 10, 11, and 12), the concentration of amphotericin B in serum had been measured during treatment after a minimum of 3 doses and ranged between 0.45 and 1.98 μg/ml (median, 1.02 μg/ml) 1 to 2 h after administration.

HPLC assays. (i) Performance. Peak heights for spiked serum and tissue samples were linearly correlated with concentrations from 0.1 to 10 μg/ml (Pearson correlation coefficient of 0.99 for 20 values). The lowest concentration of amphotericin B detected was 0.025 μg/ml of homogenate, corresponding to 0.1 μg/g of tissue. The between-assay variability of the retention time of amphotericin B in controls was 5.8%. The mean recovery rates (ranges) obtained in spiked homogenates of tissues from control patients were 87 (81 to 102), 72 (68 to 75), 78 (72 to 82), and 89% (89 to 94%) for the liver, spleen, lungs, and kidneys, respectively. The intra-assay coefficients of variation were, respectively, 5.1, 3.8, and 2.9% for the liver and 4.6, 2.3, and 1.4% for the spleen for amphotericin B concentrations of 0.5, 5, and 10 μg/ml. The between-assay coefficients of variation were, respectively, 9.1, 4.9, and 3.5% for the liver and 7.8, 6.1, and 3.3% for the spleen.

(ii) Tissue concentrations. The concentrations of amphotericin B measured in tissues of the 13 patients are reported in Table 2. Higher concentrations were recovered from the liver and the spleen compared with the lungs and the kidneys (Fig. 1). A significant correlation ($P < 0.01$) was demonstrated between tissue concentrations and the total dose of amphotericin B-deoxycholate administered. The comparison between paired organs was additional evidence of the reproducibility of the assay. Concentrations of amphotericin B in lungs and kidneys were always comparable for the right and the left organ except in one patient (patient 9). A marked difference of amphotericin B recovery was observed between both kidneys of patient 9; this patient was the only one to have histological renal abnormalities (nephroangiosclerosis and urinary cysts). Concentrations of amphotericin B in homogenates of muscle (measured in six patients) and fat (two patients) were less than 1 μg/g of tissue; these concentrations ranged between 1 and 2 μg/g in the esophagus (three patients) and small bowel (one patient) and between 0.4 and 3.1 μg/g in the thyroid (six patients). The total amount of amphotericin B recovered in each organ was calculated by taking into account the concentration and the weight of the organ. The recovery of amphotericin B from each organ calculated as the percentage of the total dose of amphotericin B-deoxycholate seemed unrelated to the total dose but characteristic for each organ (Table 3). The highest recovery was observed in the liver and ranged from 17.5 to 40.3% of the total dose. Recoveries calculated for heart, brain, pancreas, esophagus, and thyroid accounted for less than 1%. It is important to stress that patient 1 had invasive renal candidiasis despite a higher concentration of amphotericin B

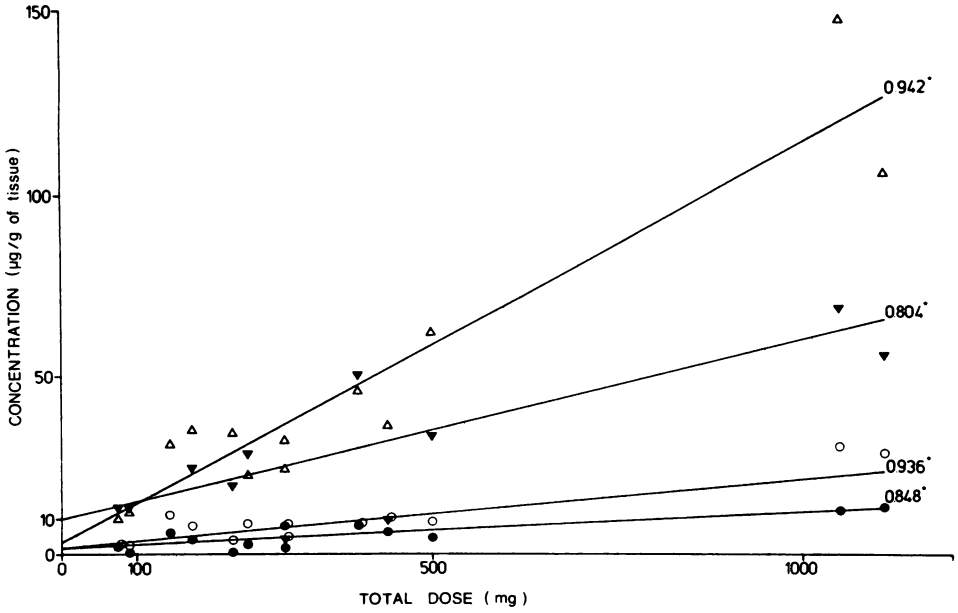

FIG. 1. Concentrations of amphotericin B in tissues of 13 patients. Each point corresponds to the mean concentration (determined by HPLC) in the tissue. Symbols: *, Pearson correlation coefficient; △, liver; ▼, spleen; ○, kidney; ●, lung.

TABLE 3. Mean recovery[a] of amphotericin B by HPLC in 13 patients

Organ	Mean recovery	SD	Range
Liver	27.5	6.4	17.5–40.3
Spleen	5.2	4.4	0.7–15.6
Lungs	3.2	3.3	0.4–13.0
Kidneys	1.5	1.0	0.6–4.1
Heart	0.4	0.4	0–1.4
Brain	0.3	0.2	0–1.4
Pancreas	0.2	0.2	0.1–0.6

[a] Results are expressed as percentages of the total dose administered. Total recovery was 38.8 ± 8.2% (range, 23.0 to 51.3%).

in the kidneys (3.3 μg/g of tissue, corresponding to 4.2% of the total dose administered) than the other patients studied.

The differences (mean; range in minutes) between sample and control retention times were as follows: liver (−0.017; −0.09 to 0.07), spleen (0.02; −0.04 to 0.15), lung (−0.006; −0.08 to 0.09), and kidney (0.018; −0.09 to 0.13). The range of these differences corresponded to 5.2, 6.2, 5.6, and 7.2% of the mean retention times of the control for liver, spleen, lung, and kidney, respectively.

Bioactivity of amphotericin B. (i) Bioassays. Based on the corresponding standard curves, the lowest concentration of amphotericin B detected was 0.4 μg/ml for tissue homogenates and 0.2 μg/ml for methanolic extracts. The intra-assay coefficients of variation in spiked homogenates (1 and 2.5 μg/ml, respectively) were 6.8 and 5.5% for the liver and 6.4 and 6.0% for the spleen. In methanolic extracts these coefficients were, respectively, 6.2 and 8.4% for the liver and 6.8 and 7.3% for the spleen. The between-assay coefficients of variation in spiked homogenates were, respectively, 13.8 and 19% for the liver and 11.1 and 15.6% for the spleen. In methanolic extracts they were, respectively, 21.5 and 20.1% for the liver and 21.9 and 18.3% for the spleen.

Bioassays were performed for all patients except patients 3, 7, and 13. The highest concentrations of amphotericin B were observed in the liver and spleen homogenates and in corresponding methanolic extracts. Tissue concentrations measured by bioassay in homogenates represented 15 to 41% of those measured by HPLC, whereas 58 to 81% were recovered from the methanolic extracts (Fig. 2). Excellent correlation was observed between HPLC and bioassay for methanolic extracts: 0.97 (Pearson coefficient of correlation), 0.96, 0.98, and 0.99 for the liver, the spleen, the lungs, and the kidneys, respectively. In addition, values determined in the extracts were always higher than the concentrations of bioactive amphotericin B measured in the homogenates (Fig. 2). For four patients who had received total doses ranging from 75 to 229.5 mg, amphotericin B was not detectable in the homogenates of the lungs. Moreover, we observed very low concentrations of bioactive drug in the lungs and the kidneys of patients in whom invasive mycosis was demonstrated. Bioassays performed on the bile after extraction in methanol showed variable results ranging from <1.6 to 24.7 μg/ml (median, 7.3 μg/ml), independent of the total dose administered. With a median concentration in bile of 7.3 μg/ml and a daily bile excretion estimated between 50 and 1,000 ml, the daily excretion ranged from 0.4 to 7.3 mg, corresponding to 0.8 to 14.6% of a 50-mg daily dose.

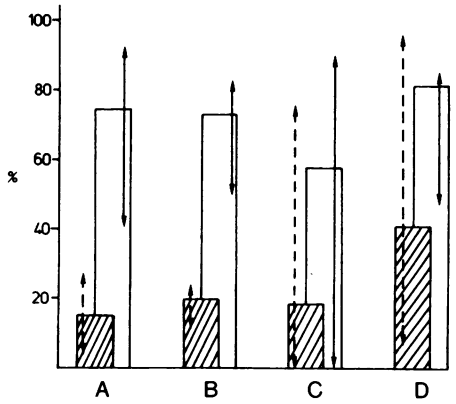

FIG. 2. Median percentages of tissue concentrations of amphotericin B determined by bioassay and expressed as comparison with HPLC (100%). Symbols: □, methanolic extracts; ▨, homogenates; ↔, range. Bars: A, liver; B, spleen; C, lung; D, kidney.

(ii) TFSs and TFCs. All tissue homogenates from the three control patients showed a reciprocal TFS of less than 2 and no TFC. The MICs determined in YNB for the three strains of *C. albicans* and *A. fumigatus* were 1.6 and 3.1 μg/ml, respectively. The minimal fungicidal concentrations were 1.6 and 6.25 μg/ml, respectively, for each organism. In YNB supplemented with 25% normal human serum, MICs were 0.4 μg/ml for all strains, and minimal fungicidal concentrations were 0.8 and 3.1 μg/ml for *C. albicans* and *A. fumigatus*, respectively. The highest TFSs were obtained in the liver, ranging from 2 to >128 (median, 64) for both fungal species. In the spleen and the lungs, the ranges of TFS were <2 to 64 (median, 16) for *C. albicans* and <2 to 16 (median, 4) for *A. fumigatus*. In the kidneys, TFSs ranged from <2 to 64 (median, 8) for *C. albicans* and from <2 to 16 (median, 4) for *A. fumigatus*. TFSs of all tissues with histologically proven invasive mycosis were low, ranging from 2 to 8 for *C. albicans* and 2 to 4 for *A. fumigatus*. Figure 3 shows the significant correlation (Spearman, $P < 0.01$) between reciprocal tissue TFSs obtained in homogenates and concentrations of amphotericin B measured by HPLC. Similarly, the correlation between bioassay of homogenates and TFS was also statistically significant for *C. albicans* and *A. fumigatus* (Spearman, $P < 0.05$), except in the lungs ($P > 0.1$).

The reciprocal TFCs for all tissue homogenates were generally below 2 for all fungal strains tested. However, TFCs ranging from 2 to 8 for *C. albicans* were observed in the liver homogenates from patients 9, 10, 12, and 13 and in the spleen homogenates from patient 9. Tissues with detectable TFCs had high concentrations of amphotericin B measured by HPLC (range, 35.6 to 147.1 μg/g; median, 50.2 μg/g). Against *A. fumigatus*, only the liver homogenate from patient 9 had detectable TFC of 2.

(iii) Correlation between expected and observed TFSs. By using the MICs measured in YNB, the following Pearson coefficients of correlation were obtained: for *C. albicans* strain 1, 0.73 ($P < 0.001$); for strain 2, 0.85 ($P < 0.001$); for strain 3, 0.74 ($P < 0.001$). The following Pearson coefficients were obtained by using MICs measured in YNB supplemented with serum: for *C. albicans* strain 1, 0.74 ($P < 0.001$); for strain 2, 0.85 ($P < 0.001$); for strain 3, 0.71 ($P < 0.001$). The slopes of the correlation lines (measured TFS versus expected TFS) were 0.37 (strain 1), 0.43 (strain 2), and 0.18 (strain 3) when the expected TFS was calculated by using the MICs measured in YNB plus serum. These slopes actually measured the proportion of amphotericin B present in the sample which remains bioactive. It appeared that only 18 to 43% of amphotericin B was bioactive in the tissues.

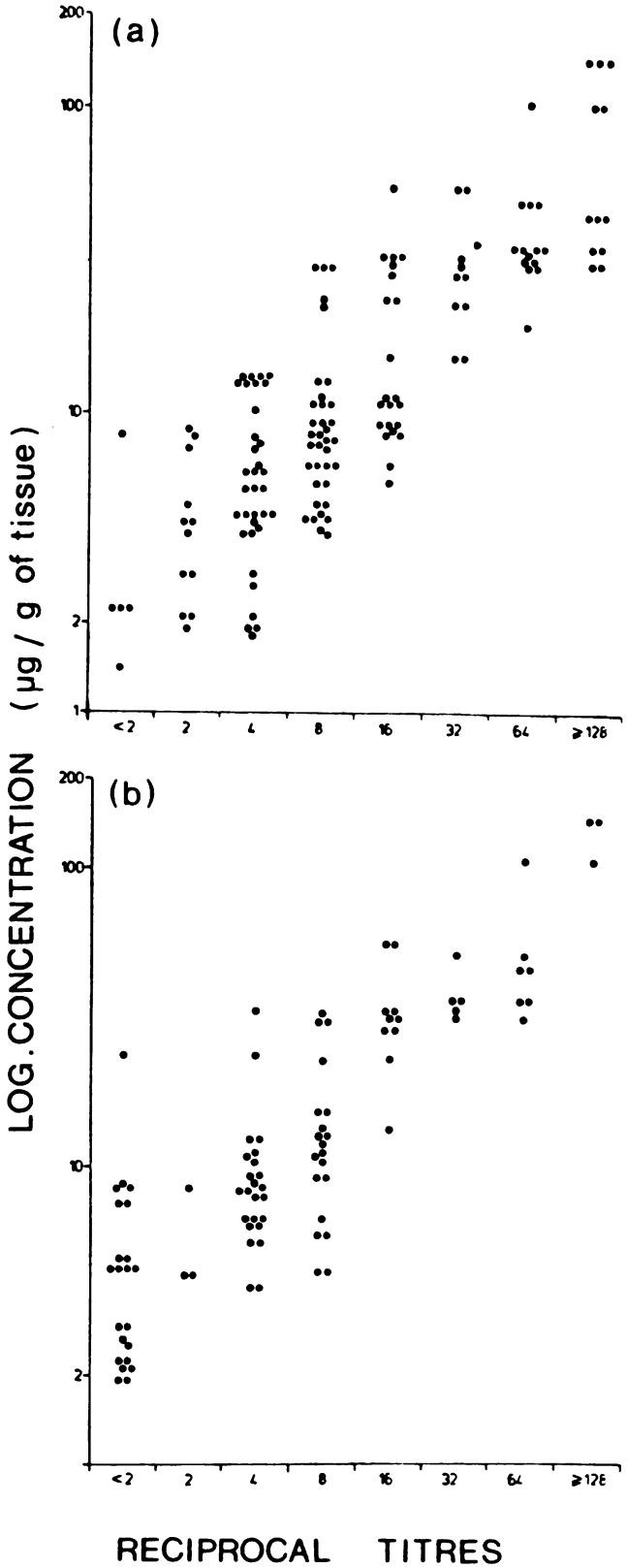

FIG. 3. Correlation between tissue concentrations of amphotericin B (determined by HPLC) and tissue TFSs for *C. albicans* (a) and *A. fumigatus* (b). Numbers of samples for reciprocal titers for *C. albicans* were <2 (n = 5), 2 (n = 12), 4 (n = 34), 8 (n = 36), 16 (n = 22), 32 (n = 11), 64 (n = 14), and ≥128 (n = 12). The corresponding values of n for *A. fumigatus* were 22, 3, 24, 20, 10, 5, 7, and 3.

This range is very similar to the bioactivity estimated from the bioassay of tissue homogenates (Fig. 2).

DISCUSSION

The distribution of amphotericin B in tissues remains poorly defined. HPLC is a highly sensitive and reproducible method for measuring amphotericin B concentrations in tissues of patients treated with amphotericin B-deoxycholate. The total recovery values observed in this study were higher than those obtained in murine tissues (14) and slightly lower than those reported by others (6) in human hepatic and splenic homogenates. In the present study and in two other studies (6, 14), the highest concentrations and recoveries of amphotericin B were observed in the liver and the spleen followed by the kidneys, lungs, and heart. By contrast, amphotericin B concentration was very low in the pancreas and brain. The recovery was best correlated with the total dose of amphotericin B administered, although each tissue showed a characteristic pattern of accumulation. Only small amounts of the drug were measured in the lungs and kidneys, which are frequent sites of invasive mycosis (17, 18). The binding sites of amphotericin B to various cells remain unknown. Great affinity of the drug to ergosterol in the membranes of fungal cells (10), to cholesterol in the cell membrane of mammalian cells, and to plasma lipoproteins has been described (4) and suggests that cell membranes (outer and inner) are the sites of binding of amphotericin B. Studies of subcellular fractionation should contribute to identifying the cell components involved.

Taking into account the concentrations of amphotericin B measured in the liver, spleen, kidneys, lungs, heart, brain, and pancreas, we recovered a mean of 38.3% (range, 23.0 to 51.3%) of the total dose administered. Lopez-Berestein et al. (13) reported that the concentrations in the lungs were 10 times higher in mice infected with *C. albicans* than in healthy animals. Comparing the fraction of the total dose recovered from the pulmonary and renal homogenates from patients with invasive mycosis with those measured in uninfected tissues, we also observed a greater recovery in infected patients than in uninfected patients. Tumoral infiltration had no significant influence on the recovery of amphotericin B in the liver, spleen, kidneys, and lungs. The presence of invasive mycosis at autopsy in tissues with very low concentrations of amphotericin B was expected. For all patients with documented candidiasis or aspergillosis, the concentration of amphotericin B (measured by HPLC) was higher than the MIC for the corresponding pathogen, suggesting that only a small part of the amphotericin B present in the tissue has an antifungal activity.

Amphotericin B concentration measured by bioassay on homogenates represented only 20 to 40% of the extractable drug measured by HPLC. The binding of amphotericin B to tissue components seems partially reversible, since in methanolic extracts the recovery measured by bioassay was 60 to 80% of that measured by HPLC. However, it has been shown that amphotericin B maintains its biological activity after in vitro binding to cholesterol and lipoproteins (4). Whether this binding, as reproduced in vitro, is identical to the binding observed in vivo remains to be investigated.

Metabolism and bioinactivation through sequestration are the possible causes for the major discrepancy between amphotericin B concentrations measured by HPLC after methanol extraction and those measured by bioassay of homogenates. Metabolism of amphotericin B is an improbable explanation for the following reasons. First, the reten-

tion time of the peak identified as amphotericin B from the samples was not different than the retention time of an authentic standard. It is very unlikely that a metabolite would have an identical retention time, having lost most of the microbiological activity and retained the characteristic absorption wavelength of intact amphotericin B. Metabolism is usually recognized by a change in retention time when the chromophore is not affected or eventually not recognized at all when the chromophore is affected. Second, up to 80% of the amphotericin B measured by HPLC was also found to be bioactive when the bioassays were performed on methanolic extracts, suggesting that amphotericin B present in the tissue samples is strongly bound, presumably to the cholesterol of the cell membranes. Third, amphotericin B is unaffected by incubation with rat liver microsomes (6) or with human liver extracts (the present study).

Very low or undetectable concentrations of bioactive amphotericin B (<5 μg/g of tissue) in tissues with documented invasive mycosis could contribute to explaining the presence of viable fungi, despite extractable drug concentrations that were occasionally higher than the MIC for the corresponding pathogen. However, there are several other possible explanations for the coexistence of viable fungi with relatively high concentrations of amphotericin B in the tissues. The yeast cells in the tissues are in different physiological and metabolic states than those tested in vitro; moreover, the local environment at the site of infection may impair the mechanism of killing of amphotericin B.

The present study also evaluated TFSs and TFCs in tissue homogenates to measure the bioactivity of amphotericin B. Whether this method with various pathogenic strains may be more appropriate for the management of each patient than a bioassay performed with a nonpathogenic filamentous fungus is unknown. Although a majority of tissue homogenates did not show any fungicidal activity, TFCs correlated with the concentration of amphotericin B and with the total dose administered. Further studies are necessary to establish whether those TFCs are predictive of the outcome of treatment.

The present study is the first to report measures of amphotericin B concentrations in the bile of humans, confirming data obtained by Craven et al. (7) and Lawrence et al. (11), who have suggested in their animal models that the bile could be a significant route of excretion.

This study indicates that only a small proportion of amphotericin B administered as amphotericin B-deoxycholate seems bioavailable in tissues despite high concentrations of the drug in several of them as detected by HPLC. This observation and the fact that organs frequently identified as the sites of invasive mycosis exhibit relatively low concentrations of the drug may help explain the frequent therapeutic failures. The use of amphotericin B-deoxycholate in doses higher than 1.2 mg/kg per day is limited by a low therapeutic index. These observations have recently stimulated the interest in the development of new modalities for antifungal drug delivery such as amphotericin B incorporated into liposomes (12, 13, 20; F. Meunier, Rev. Infect. Dis., in press; F. Meunier, J. P. Sculier, A. Coune, C. Brassinne, C. Heymans, C. Laduron, N. Collette, C. Hollaert, D. Bron, and J. Klastersky, Ann. N.Y. Acad. Sci., in press). This approach is extremely encouraging and may change the pharmacological properties of amphotericin B, resulting in better tolerance by the treated patients as well as in the improvement of the therapeutic index of this antifungal agent.

ACKNOWLEDGMENT

F.M. is a Research Associate supported by the Fund for Medical Scientific Research, Belgium.

LITERATURE CITED

1. **Atkinson, A. J., and J. E. Bennett.** 1978. Amphotericin B pharmacokinetics in humans. Antimicrob. Agents Chemother. **13**:271–276.
2. **Bennett, J. E.** 1985. Antifungal agents, p. 263–270. *In* G. C. Mandell, G. R. Douglas, and J. E. Bennett (ed.), Principles and practice of infectious diseases, 2nd ed. John Wiley & Sons, Inc., New York.
3. **Bindschadler, D., and J. Bennett.** 1969. A pharmacologic guide to the clinical use of amphotericin B. J. Infect. Dis. **120**:427–436.
4. **Brajtburg, J., S. Elberg, J. Bolard, G. S. Kobayashi, R. A. Levi, R. E. Ostlund, D. Schlessinger, and G. Medoff.** 1984. Interaction of plasma proteins and lipoproteins with amphotericin B. J. Infect. Dis. **149**:986–997.
5. **Brassinne, C., C. Laduron, A. Coune, J. P. Sculier, C. Hollaert, N. Collette, and F. Meunier.** 1987. High-performance liquid chromatographic determination of amphotericin B in human serum. J. Chromatogr. **419**:401–407.
6. **Christiansen, K. J., E. M. Bernard, J. W. M. Gold, and D. A. Armstrong.** 1985. Distribution and activity of amphotericin B in humans. J. Infect. Dis. **152**:1037–1043.
7. **Craven, P. C., T. M. Ludden, D. J. Drutz, W. Rogers, K. A. Haegele, and H. B. Skrdlant.** 1979. Excretion pathways of amphotericin B. J. Infect. Dis. **140**:329–341.
8. **Drutz, D.** 1982. Newer antifungal agents and their use, including an update on amphotericin B and flucytosine, p. 97–135. *In* J. S. Remington and M. N. Swartz (ed.), Current clinical topics in infectious diseases, vol. 3. McGraw-Hill Book Co., New York.
9. **Horn, R., B. Wong, T. E. Kiehn, and D. A. Armstrong.** 1985. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. Rev. Infect. Dis. **7**:646–653.
10. **Kotler-Brajtburg, J., G. Medoff, D. Schlessinger, and G. S. Kobayashi.** 1974. Characterization of the binding of amphotericin B to *Saccharomyces cerevisiae* and relationship to the antifungal effects. Antimicrob. Agents Chemother. **6**:770–776.
11. **Lawrence, R. M., P. D. Hoeprich, F. A. Jagdis, N. Monji, A. C. Huston, and C. P. Schaffner.** 1980. Distribution of doubly radiolabelled amphotericin B methyl ester and amphotericin B in the non-human primate, *Macaca mulatta*. J. Antimicrob. Chemother. **6**:241–249.
12. **Lopez-Berestein, G., G. P. Bodey, L. S. Frankel, and K. Mehta.** 1987. Treatment of hepatosplenic fungal infections with liposomal amphotericin B. J. Clin. Oncol. **5**:310–317.
13. **Lopez-Berestein, G., M. G. Rosenblum, and R. Mehta.** 1984. Altered tissue distribution of amphotericin B by liposomal encapsulation: comparison of normal mice to mice infected with *Candida albicans*. Cancer Drug Delivery **1**:199–205.
14. **Mayhew, J. W., C. Fiore, T. Murray, and M. Barza.** 1983. An internally-standardized assay for amphotericin B in tissues and plasma. J. Chromatogr. **274**:271–279.
15. **Meunier, F.** 1986. Fungistatic and fungicidal activity in volunteers receiving antifungal agents. Eur. J. Clin. Microbiol. **5**: 103–109.
16. **Meunier-Carpentier, F., T. Kiehn, and D. A. Armstrong.** 1981. Fungemia in the immunocompromised host: changing patterns, antigenemia, high mortality. Am. J. Med. **71**:363–370.
17. **Myerowitz, R. L.** 1983. Localized and disseminated candidiasis, p. 95–114. *In* R. L. Myerowitz (ed.), The pathology of opportunistic infections. Raven Press, New York.
18. **Myerowitz, R. L.** 1983. Invasive pulmonary and disseminated aspergillosis, p. 115–128. *In* R. L. Myerowitz (ed.), The pathology of opportunistic infections. Raven Press, New York.
19. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemo-

ther. **18**:699–708.

20. **Sculier, J. P., A. Coune, F. Meunier, C. Brassinne, C. Laduron, C. Hollaert, N. Collette, C. Heymans, and J. Klastersky.** 1988. Pilot study of amphotericin B entrapped into sonicated liposomes in cancer patients with fungal infections. Eur. J. Cancer Clin. Oncol. **24**:527–538.

21. **Shadomy, S., A. Espinell-Ingroff, and R. Cartwright.** 1985. Laboratory studies with antifungal agents: susceptibility tests and bioassays, p. 991–999. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.