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# Evolving Role of Flucytosine in Immunocompromised Patients: New Insights into Safety, Pharmacokinetics, and Antifungal Therapy

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Flucytosine is an antifungal agent useful in combination with amphotericin B in the treatment of several deeply invasive mycoses. The potentially dose-limiting, hematologic, gastrointestinal, and hepatic toxicities of flucytosine lead to a reluctance to use it in myelosuppressed patients. To investigate the safety and tolerability of flucytosine in this setting, we evaluated its use in 17 patients with cancer or aplastic anemia during a 2½-year period at our institution and reviewed the literature describing mechanisms of action, resistance, in vitro and in vivo antifungal activity, clinical antifungal activity, pharmacokinetics, and toxicity. The combination of amphotericin B plus flucytosine eradicated the mycosis in 12 (71%) of 17 patients, whereas 3 (18%) of 17 died of progressive fungal infection. Serial serum levels of flucytosine measured by a creatinine iminohydrolase assay permitted reliable dosage adjustment. During therapy, only 2 (12%) of 17 patients had elevated mean serum levels of flucytosine (>100 µg/mL) and 3 (18%) other patients had transiently elevated levels. Paired serum samples ( $n = 45$ ) obtained at steady state during therapy with orally administered flucytosine showed similar peak and trough levels. Adverse effects of flucytosine therapy included one case each of reversible nausea, diarrhea, elevated transaminase levels, and thrombocytopenia. No cases of bone marrow aplasia, enterocolitis, hepatitis, or death due to flucytosine toxicity were encountered. We conclude that flucytosine in combination with amphotericin B is well tolerated in myelosuppressed patients when serum flucytosine levels are serially monitored.

Flucytosine (5-FC) is a water-soluble, fluorinated pyrimidine analog that was discovered in 1957 during the search for antineoplastic agents. Its chemical structure is related to that of 5-fluorouracil and floxuridine. In the United States, it is administered primarily orally; in Europe, intravenous preparations are more widely available. The activity of flucytosine against murine cryptococcosis was reported by Grunberg et al. in 1964 [1]. Tassel and Madoff [2] and Utz et al. [3] first used flucytosine in humans to treat candidiasis and cryptococcosis in 1968. The antifungal capability of flucytosine is the sole basis for its present clinical usage.

During the last several years, as a result of the dramatic increase in fungal infections in immunocompromised hosts, the dose-limiting nephrotoxicity of amphotericin B, the paucity of readily available, effective antifungal compounds, and the improved therapeutic outcome in certain mycoses with combination antifungal therapy, interest in using flucytosine together with amphotericin B has increased.

During its early clinical use, however, flucytosine was associated with potentially fatal bone marrow aplasia [4, 5], hepatic necrosis [6], and intestinal perforation [7, 8]. Retrospective analysis indicates that these observations were made

either in the absence of direct measurements of serum flucytosine levels or in patients whose flucytosine levels were >100 µg/mL and even 125 µg/mL [9] for a prolonged period, usually as a consequence of amphotericin B-induced renal insufficiency. In view of this toxicity, clinicians have been reluctant to use flucytosine in the treatment of invasive mycoses in immunocompromised patients, especially those with AIDS in fear of additive granulocytopenia or thrombocytopenia, hepatotoxicity, and gastrointestinal adverse effects.

To evaluate the safety and tolerability of flucytosine in immunocompromised patients, we reviewed the clinical courses and flucytosine levels of 21 consecutive patients with invasive mycoses who were treated with the combination of amphotericin B and flucytosine. We investigated the frequency of adverse effects attributable to flucytosine, the correlation, if any, between flucytosine levels and toxicity, and the influence of such effects on the continuation of flucytosine therapy. We further reviewed the literature describing the properties, use, safety, and tolerability of flucytosine.

## Patients and Methods

During a 2½-year period (January 1987 to July 1989), 21 patients followed by the Infectious Diseases Section of the National Cancer Institute were treated with flucytosine in combination with amphotericin B for invasive fungal infections. All were inpatients at the National Institutes of Health (NIH) Clinical Center when a diagnosis of deep fungal infec-

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tion was made on the basis of a positive culture of blood or deep tissue with compatible clinical findings or with histopathologically demonstrable organisms. For patients with initially positive cultures of blood or pleural fluid, culture was repeated on multiple occasions. In patients with hepatosplenic candidiasis, however, liver biopsy was not repeated but clinical resolution of infection was based on defervescence and radiographic resolution or calcification of lesions. All patients were observed for recurrence of fungal infection during subsequent hospitalizations.

### Drug-Related Toxicity

Adverse drug effects were attributed to flucytosine only if the effects developed after institution of therapy with the drug and improved after its administration was discontinued and if there was no other immediate explanation for their occurrence. The decision to ascribe toxicity to flucytosine was based on the judgment of the patients' primary physicians, who then lowered the dosage of the drug or discontinued its administration.

Granulocytopenia was defined as a reduction of the absolute granulocyte count to  $<500/\text{mm}^3$ , and thrombocytopenia was defined as a reduction of the platelet count to  $<100,000/\text{mm}^3$ . Hepatotoxicity was defined as a rise in transaminase, bilirubin, or alkaline phosphatase levels to three or more times the upper limits of normal. Gastrointestinal toxicity was defined as the new onset of nausea, vomiting, diarrhea, or enterocolitis without other cause.

### Method of Flucytosine Assay

Blood samples were routinely obtained for assay of flucytosine levels immediately before and 30 minutes to 1 hour after administration of flucytosine as part of general patient management; these assays were not used to delineate the specific pharmacokinetic properties of flucytosine (e.g., volume of distribution, half-life, and area under the concentration curve). In the event of a suspected adverse effect attributable to flucytosine, additional samples were assayed for drug levels and assessed for toxicity. All but one of the assays were performed in the NIH clinical chemistry laboratory with use of a modification [10] of the creatinine iminohydrolase assay [11–14]. In brief, in this assay flucytosine is hydrolyzed in the presence of creatinine iminohydrolase (from *Flavobacterium filamentosum* E. C. 35421; Eastman Kodak, Rochester, NY), and dissolved in 5% bovine albumin and PBS, pH 7.5, to form the ammonium ion and 5-fluorouracil. The ammonium ion is subsequently oxidized by glutamate dehydrogenase in the presence of 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH) to form free ammonia ( $\text{NH}_3$ ) and NAD. The reactions are automatically coupled in a Cobas Bio centrifugal analyzer (Roche Analytical, Nutley, NJ). The liberation of ammonia is measured by the differ-

ence in spectrophotometric absorbance ( $\Delta A$ ) between the serum sample and a blank control. The concentration of creatinine  $S_{cr}$  is determined with use of the Jaffé reaction, and the concentration of flucytosine is determined thus:  $[5\text{-FC}] = 0.573 \times [\Delta A - 2.062 (S_{cr}) - 10]$ . A comparison with an HPLC method showed good agreement:  $y = 0.98x + 1.34$  mg/L,  $Sy \cdot x = 3.7$  mg/L, as analyzed with the Deming de-biased regression ( $n = 37$ ). This assay had good precision, with coefficients of variation of  $<3\%$  for within-run controls and  $<6\%$  for between-run controls. A level of flucytosine was considered toxic if it was  $>100 \mu\text{g/mL}$ .

In patients with normal renal function, the initial dosage of flucytosine was 37.5 mg/kg every 6 hours. In patients with a creatinine clearance of  $<40$  mL/minute, the dosage was decreased to 37.5 mg/kg every 12 hours, and in those with a creatinine clearance of  $<20$  mL/minute, the dosage was 37.5 mg/kg every 24 hours or after dialysis when required. In addition, doses of flucytosine were adjusted to maintain serum levels at  $<100 \mu\text{g/mL}$  in all patients. Mean flucytosine levels before and after a dose were calculated at steady state for each patient in this study by summing the serum values and dividing by the total number of serum samples obtained.

## Results

### Patient Population

Four patients were excluded from analysis: three had been treated with flucytosine for  $<1$  week, and the entire chart of one patient was unavailable for review. There were 17 evaluable patients (9 males and 8 females). Nine had lymphoma, including the lymphoblastic type, three had solid tumors, three had leukemia, and one had aplastic anemia. At the time of diagnosis of the fungal infection, 14 patients were being actively treated with combination chemotherapy regimens, one patient was being treated with single-agent palliative chemotherapy for end-stage breast carcinoma, and two were receiving no chemotherapy.

### Microbiology

*Candida albicans* was the infecting organism in four patients, producing a fasciitis, fungemia, hepatosplenic candidiasis, and combined fungemia and hepatosplenic candidiasis in one patient each (table 1). An unidentified *Candida* species was responsible in four cases—three of hepatosplenic candidiasis and one of fungemia. *Torulopsis glabrata* and *Candida guilliermondii* were each responsible for one episode of fungemia. *Candida tropicalis* was found in two cases of fungemia and in one case of hepatosplenic candidiasis. *Cryptococcus neoformans* was found in two patients, one with a progressive solitary lung nodule and the other with a

**Table 1.** Profiles of patients treated with amphotericin B (AmB) and flucytosine (5-FC).

Patient no. (age/sex)	Primary disease	Chemotherapy*	Days ANC <sup>†</sup> <500	Indication for administration of 5-FC		Outcome of infection
				Fungal infection		
1 (41/M)	AIDS/Hodgkin's disease	ABVD	24	<i>C. albicans</i> fasciitis <sup>‡</sup>	Development or progression during AmB	Resolution
2 (62/M)	Nodular histiocytic lymphoma	PMACE, cytarabine, bleomycin, vincristine	10	<i>Aspergillus</i> pneumonia <sup>§</sup>	Development or progression during AmB	Resolution
3 (23/F)	Refractory T cell lymphoma	Etoposide, cisplatin	10	<i>T. glabrata</i> fungemia <sup>‡</sup>	Development or progression during AmB	Resolution
4 (39/F)	Breast carcinoma	Vinblastine	1	<i>C. guilliermondii</i> fungemia	Prevention of emergence of polyene resistance	Resolution
5 (45/F)	Adult T cell leukemia	Vincristine, cyclophosphamide, prednisone, AraC, VM-26, IT methotrexate	18	<i>C. albicans</i> fungemia and hepatosplenic candidiasis <sup>‡</sup>	Development or progression during AmB	Death, NEI <sup>  </sup>
6 (24/M)	s/p Hodgkin's disease	None	0	<i>C. neoformans</i> lung nodule	Combination therapy for cryptococcosis	Resolution
7 (39/M)	AIDS/Burkitt's lymphoma	Cyclophosphamide, Ara-C, etoposide, vincristine, IT methotrexate	12	<i>C. tropicalis</i> fungemia <sup>§</sup>	Development or progression during AmB	Death
8 (18/M)	Lymphoblastic lymphoma	Cyclophosphamide, AraC, VM-26, vincristine, pentostatin, prednisone, IT methotrexate	30	<i>C. albicans</i> fungemia and hepatosplenic candidiasis <sup>‡</sup>	Development or progression during AmB	Death, NEI <sup>  </sup>
9 (5/F)	Acute lymphoblastic leukemia	Vincristine, Ad, prednisone, methotrexate, L-asparaginase, cyclophosphamide, Ara-C	21	<i>C. tropicalis</i> fungemia and hepatosplenic candidiasis	Improved activity against <i>C. tropicalis</i>	Resolution
10 (22/M)	Lymphoblastic lymphoma	Cyclophosphamide, Ara-C, vincristine, VM-26, prednisone, methotrexate, IT methotrexate, pentostatin	37	<i>C. tropicalis</i> fungemia	Improved activity against <i>C. tropicalis</i>	Resolution
11 (47/F)	Ovarian carcinoma	Cyclophosphamide, cisplatin, CBDCA	11	<i>C. albicans</i> fungemia <sup>‡</sup>	Development or progression during AmB	Resolution
12 (22/M)	Acute promyelocytic leukemia	Ad, etoposide, Ara-C, thioguanine, dexamethasone	19	Hepatosplenic candidiasis	Combination therapy for treatment of hepatosplenic candidiasis	Resolution
13 (48/F)	Ovarian carcinoma	Cyclophosphamide, cisplatin, CBDCA	23	Hepatosplenic abscess	Combination therapy for treatment of hepatosplenic candidiasis	Receiving therapy
14 (20/F)	Lymphoblastic lymphoma	Cyclophosphamide, Ad, vincristine, prednisone, L-asparaginase, IT methotrexate, IT AraC	26	<i>Candida</i> fungemia and hepatosplenic candidiasis <sup>§</sup>	Development or progression during AmB	Switched to liposomal amphotericin B, death, NEI
15 (38/F)	Aplastic anemia	GM-CSF	>12 y	<i>A. fumigatus</i> pneumonia and myositis <sup>§</sup>	Development or progression during AmB	Death
16 (58/M)	Diffuse, undifferentiated lymphoma	PMACE, Ara-C, vincristine	13	<i>C. tropicalis</i> fungemia <sup>§</sup>	Development or progression during AmB	Death
17 (34/M)	Adrenal carcinoma	Etoposide, Ad, amiodarone, suramin	3	<i>C. neoformans</i> fungemia and empyema	Combination therapy for cryptococcosis	Resolution

\* Treatment regimens immediately before diagnosis of fungal infection: ABVD = Adriamycin (doxorubicin), bleomycin, vinblastine, dacarbazine; PMACE = prednisone, methotrexate, anthracycline derivative; AraC = cytarabine; VM-26 = teniposide; Ad = anthracycline derivative; CBDCA = carboplatin, bleomycin, dexamethasone, AraC; GM-CSF = granulocyte-macrophage colony-stimulating factor; IT = intrathecal.

<sup>†</sup> Absolute neutrophil count.

<sup>‡</sup> Patient was already receiving AmB (0.5 mg/[kg · day]) when 5-FC was added to regimen.

<sup>§</sup> Patient was already receiving AmB (1.0 mg/[kg · day]) when 5-FC was added to regimen.

<sup>||</sup> No evidence of infection ante mortem or at autopsy.

pleural effusion and cryptococcemia. *Aspergillus* species were responsible for two cases of pneumonia.

### Indications for Flucytosine Therapy

Of the 17 patients (table 1), five were treated with flucytosine after having developed a fungal infection during treatment with a standard empirical regimen of amphotericin B (0.5 mg/[kg · day]), and three received flucytosine for a fungal infection that developed or worsened during treatment with high doses of amphotericin B (0.75–1.0 mg/[kg · day]). Two patients received amphotericin B and flucytosine from the outset for cryptococcosis. The remaining seven patients received the combination regimen from the time of diagnosis for *C. tropicalis* fungemia (two), hepatosplenic candidiasis (two), aspergillosis (two), and *C. guilliermondii* fungemia (one).

### Outcome

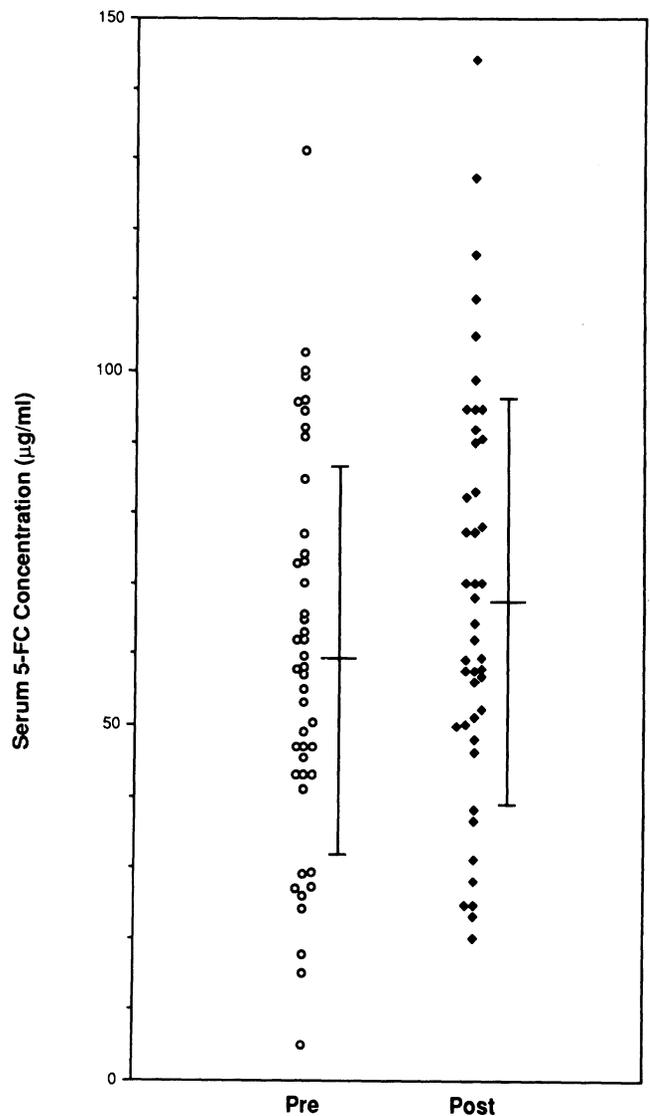
In 10 of 17 patients, the combination of amphotericin B and flucytosine eradicated the fungal infection, and the patient survived (table 1). Six patients died, three as a direct result of the infection and three as a result of supervening conditions; one of these latter patients required a change in therapy to a liposomal preparation of amphotericin B. One patient with hepatosplenic candidiasis is still receiving antifungal agents. Treatment with the combination of flucytosine plus amphotericin B eradicated the fungal infection in six of 10 patients whose infection developed or progressed during treatment with amphotericin B alone. The combination of amphotericin B plus flucytosine eradicated the mycosis in 12 (71%) of 17 patients.

### Safety and Tolerance

Twelve patients were treated with oral flucytosine, and five were treated with the intravenous preparation because of oral endotracheal intubation. Results of assays of flucytosine levels were readily obtainable within 48 hours for all patients. Forty-five paired samples for drug-level determinations were obtained from the 12 patients before and after oral flucytosine administration. There were only slight differences in pre- and post-dose flucytosine levels (figure 1). Differences between pre- and post-dose flucytosine levels after intravenous administration of flucytosine were greater than those after oral administration.

Table 2 depicts the profile of toxic reactions attributed by the clinicians caring for the patients, with the mean serum levels of flucytosine before (trough) and after (peak) the dose. No toxicity was attributable to flucytosine in 13 of 17 patients. Only two patients had mean flucytosine levels of >100 µg/mL during treatment, and neither developed toxic reactions.

Nine of the 17 patients were granulocytopenic at the in-



**Figure 1.** Scatter plot of paired serum concentrations of flucytosine (5-FC;  $n = 45$ ) before (left) and 1 hour after (right) oral administration. Difference between means was not statistically significant.

ception of flucytosine therapy. Flucytosine was temporarily discontinued in one of these nine patients because of prolonged granulocytopenia after recent intensive chemotherapy for lymphoblastic lymphoma. The level of flucytosine was determined at an outside laboratory and was not available for review. Flucytosine therapy was later reinstated and continued for >2 months, with complete resolution of hepatosplenic candidiasis and without further complications.

Eight of the 17 patients were not granulocytopenic at the onset of flucytosine therapy. Flucytosine did not produce granulocytopenia in these eight patients. One of 17 patients developed prolonged thrombocytopenia (table 2) that was attributed to recurrent acute T cell leukemia, septic shock, and consumptive coagulopathy. Nonetheless, flucytosine

**Table 2.** Mean serum levels of flucytosine before and after dosing and toxicities associated with flucytosine.

Patient no.	Total AmB dose (g)	Days of 5-FC	5-FC levels (mean $\pm$ SEM; $\mu\text{g}/\text{mL}$ )		Maximal 5-FC level ( $\mu\text{g}/\text{mL}$ )	Change in 5-FC dose (reason)	Hepato-toxicity	Gastro-intestinal toxicity	Myelosuppression	CNS toxicity
			Trough	Peak						
1	1	14	45 $\pm$ 2	44 $\pm$ 6	50	No	—	—	—	—
2	2	16	101 $\pm$ 27	75.3 $\pm$ 17.6	128	Yes (initially low levels)	—	—	—	—
3	2	37	48.9 $\pm$ 2.4	79 $\pm$ 3.6	93.4	No	—	—	—	—
4	0.5	14	90	None	90	Yes (initially high levels)	—	—	—	—
5	3	48	37.8 $\pm$ 3.7	70.1 $\pm$ 2.3	101	Yes (initially high levels and thrombocytopenia)	—	—	Thrombocytopenia	—
6	1	66	66*	None	66	No	—	—	—	—
7	0.5	7	<5	20	20	Yes (elevated BUN and Cr)	—	—	—	—
8	4	94	64	96.5 $\pm$ 0.5	97	Yes (pancytopenia due to chemotherapy)	—	—	—	—
9	1.55	140	49.7 $\pm$ 3.7	62.6 $\pm$ 4.7	77.3	No	—	—	—	—
10	3	56	34 $\pm$ 7.2	60.8 $\pm$ 9.9	83	Yes (hepatic dysfunction)	Elevated liver enzymes	—	—	—
11	2.5	24	48.3 $\pm$ 3.2	73.6 $\pm$ 4.4	92	Yes (elevated BUN and Cr)	—	—	—	—
12	5	62	90.2 $\pm$ 3.8	96.2 $\pm$ 5.4	127	Yes (initially elevated 5-FC levels and elevated BUN and Cr)	—	Nausea	—	—
13	3.4	180	50.3 $\pm$ 7.8	53 $\pm$ 7.5	78	Yes (diarrhea)	—	Diarrhea	—	—
14	>5	49	55.3 $\pm$ 6.7	49.4 $\pm$ 4.8	73	Yes (elevated BUN and Cr)	—	—	—	—
15	2.5	67	72.2 $\pm$ 23.3	78 $\pm$ 18.8	105	Yes (initially elevated 5-FC levels)	—	—	—	—
16	2	12	55.2 $\pm$ 4.7	58 $\pm$ 2.4	65	Yes (elevated BUN and Cr)	—	—	—	—
17	2.1	60	112 $\pm$ 19.5	117 $\pm$ 27	144	Yes (elevated 5-FC levels)	—	—	—	—

NOTE. AmB = amphotericin B; 5-FC = flucytosine; BUN = blood urea nitrogen; Cr = creatinine; — = none.

\* Determination performed at an outside laboratory.

was discontinued despite pre-dose levels of 37.8  $\mu\text{g}/\text{mL}$  and post-dose levels of 70.1  $\mu\text{g}/\text{mL}$ . The patient later died of progressive multisystem failure due to the above mentioned processes but had no evidence of fungal infection immediately ante mortem.

Hepatotoxicity with elevated levels of aspartate and alanine aminotransferase (AST and ALT), alkaline phosphatase, and total bilirubin was attributed to flucytosine in one of 17 patients (table 2). This patient, who developed *C. tropicalis* fungemia after autologous bone marrow transplantation for lymphoblastic lymphoma, had hepatic dysfunction before the institution of flucytosine therapy: AST, 89 IU/L; ALT, 201 IU/L; alkaline phosphatase, 249 IU/L; and total bilirubin, 1.1 mg/dL. All of these values increased while the patient was being treated with amphotericin B and flucytosine. Peak values were AST, 238 IU/L; ALT, 660 IU/L; alkaline phosphatase, 441 IU/L; and total bilirubin, 2.8 mg/dL. Mean flucytosine levels in this patient were 34  $\mu\text{g}/\text{mL}$  before the dose and 60.8  $\mu\text{g}/\text{mL}$  after the dose (table 2). Flucytosine was discontinued for 4 days. When treatment with the same dose was reinstated, hepatic enzyme and total bilirubin values remained elevated, but the patient eventually completed a full course of amphotericin B plus flucytosine, with complete resolution of the fungemia. The liver

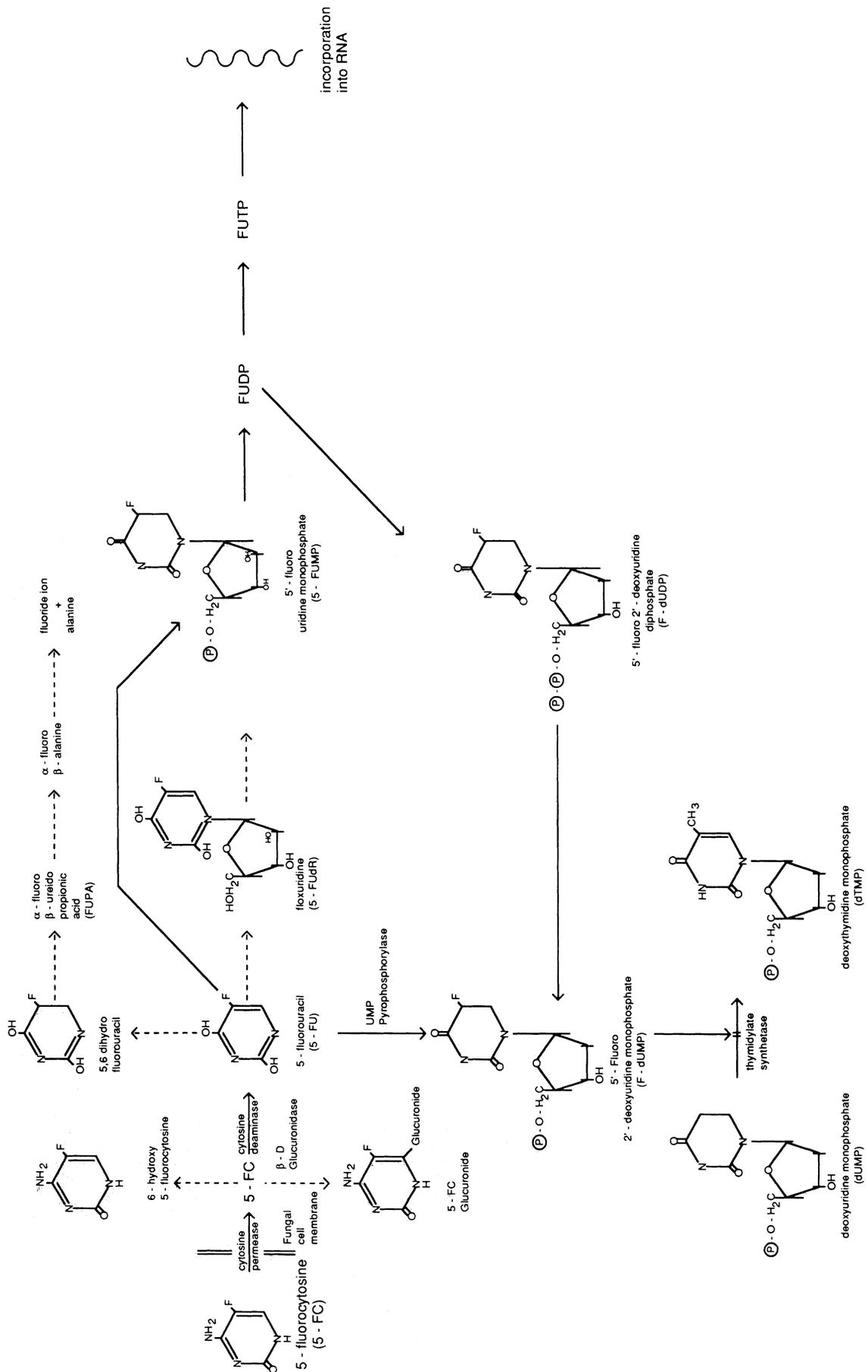
function values all normalized after antifungal therapy was discontinued.

Transient gastrointestinal symptoms manifested by nausea and diarrhea in one patient each (table 2) were possibly attributable to flucytosine and were treated symptomatically. The nausea was associated with mean levels of flucytosine of 90.2  $\mu\text{g}/\text{mL}$  before and 96.2  $\mu\text{g}/\text{mL}$  after the dose (table 1). The patient with diarrhea had been noted to have *Clostridium difficile* in her stools on two previous occasions. Flucytosine was continued, as mean levels were 50.3  $\mu\text{g}/\text{mL}$  before and 53  $\mu\text{g}/\text{mL}$  after the dose (table 1). Diarrhea recurred twice more, but the episodes were short-lived and easily managed with diphenoxylate. She is still receiving antifungal therapy with flucytosine and has had no further diarrhea. Cutaneous hypersensitivity directly attributable to flucytosine was not observed.

## Literature Review

## Mechanism of Action

After flucytosine is taken up by susceptible fungal cells by means of the fungi-specific enzyme cytosine permease, it has



two primary mechanisms of action (figure 2): (1) conversion by cytosine deaminase into 5-fluorouracil (5-FU) with subsequent conversion through several intermediates into 5-fluorouridine triphosphate and incorporation into fungal RNA with resultant inhibition of protein synthesis [15]; and (2) conversion by uridine monophosphate pyrophosphorylase into 5-fluorodeoxyuridine monophosphate (F-dUMP), which inhibits thymidylate synthetase and, consequently, DNA synthesis [19]. These two mechanisms have recently been confirmed in a study using  $^{19}\text{F}$  nuclear magnetic resonance spectroscopy [20].

### Mechanisms of Resistance

Theoretically, flucytosine resistance in fungi may arise from mutations affecting the production of uridine monophosphate pyrophosphorylase, cytosine permease, or cytosine deaminase [21, 22] (enzymes not required for survival of the fungal cell) or via increased production of pyrimidines. Defective uridine monophosphate pyrophosphorylase is the most commonly recognized source of resistance. According to Normark and Schönebeck [23], there are two phenotypes of resistance; the class 1 phenotype is total resistance to high concentrations of flucytosine, and the class 2 phenotype is initial sensitivity to flucytosine at low concentrations with the eventual development of resistance after a period of incubation. In addition, 99% of clinical strains of *C. albicans* serotype A are susceptible to flucytosine, whereas only 17% of strains of serotype B are initially susceptible [24]. It has been suggested by electron microscopic studies that the electron density of the cell wall of serotype B isolates is greater than that of serotype A, possibly accounting for this difference [25]. Since cytosine permease is not present in mammalian cells, selective uptake of the drug by susceptible fungi should occur and toxicity should be minimal. Conversely, there is evidence [26, 27] suggesting that bacteria resident in the gastrointestinal tract deaminate flucytosine into fluorouracil, a reaction resulting in myelosuppression. Moreover, Malet-Martino and colleagues [17], using  $^{19}\text{F}$  magnetic resonance spectroscopy, clearly correlated the level of intestinal gram-negative bacilli with the appearance of urinary fluorouracil metabolites, a finding lending further credence to this schema.

### Correlation Between in Vitro and in Vivo Antifungal Activity

Flucytosine has long shown efficacy against *Candida* species, *T. glabrata*, and *C. neoformans* in vitro. MICs for susceptible organisms are usually 0.5–12.5  $\mu\text{g}/\text{mL}$  [28] and are often  $<1 \mu\text{g}/\text{mL}$ . Early studies in Europe [29, 30] demonstrated a rate of in vitro resistance to flucytosine by *Candida* species of 4.1%–11.7%. Stiller et al. [31] reported a 15.5% rate of primary resistance in 402 clinical isolates of *Candida* species. Shadomy and colleagues [24] reported resistance to flucytosine in 8 of 15 clinical isolates of *C. albicans* and Marks et al. [32], in 8 of 20. *C. neoformans*, on the other hand, has shown greater susceptibility to flucytosine, with only 4% of isolates showing primary resistance [33]. In a small series by Shadomy, however, up to 24.5% of *C. neoformans* isolates were resistant [34].

Normark and Schönebeck [23] have recommended maintaining serum concentrations of flucytosine above 25  $\mu\text{g}/\text{mL}$  as one means of circumventing the development of secondary resistance. Another way is to combine flucytosine with amphotericin B—now a routine practice. Seminal work by Medoff et al. [35] demonstrated the synergistic action and fungicidal properties of flucytosine in combination with amphotericin B against isolates of *C. albicans*, *C. tropicalis*, and *C. neoformans* that had shown growth when incubated in the presence of subinhibitory concentrations of either drug alone. These results were confirmed by Hoeprich and Finn [36]. Rabinovich et al. [37], using a murine model, demonstrated greatly improved survival and a 10- to 100-fold reduction in cfu of *C. albicans* in the kidneys of animals given the combination of amphotericin B and flucytosine. Similarly, Thaler et al. [38] demonstrated significantly greater antifungal activity in the kidneys in a rabbit model of chronic disseminated candidiasis with persistent granulocytopenia.

Stiller et al. [31], using mice infected intravenously with *C. albicans*, suggested that resistance of *C. albicans* to flucytosine is not absolute and that a continuum of susceptibilities exists. These authors grouped *C. albicans* isolates into four different groups on the basis of susceptibility to flucytosine over a 7-day period. In their experience, in vitro susceptibility of *C. albicans* correlated with in vivo response to flucytosine. These findings were confirmed by Polak and Dixon [39], who were able to stratify 40 isolates of *C. albicans* in a similar fashion. Mice infected with isolates that were most susceptible to flucytosine survived significantly longer than mice infected with isolates that were highly resistant.

**Figure 2.** A review of the mechanism of action and metabolism of 5-FC. 5-FC is transported across the cell membrane by cytosine permease and once inside the cell has three possible fates: conversion by cytosine deaminase to 5-FU (major pathway); conversion to 6-hydroxy-5-fluorocytosine (minor pathway); or conversion to 5-FC glucuronide (minor pathway). 5-FU, in turn, is converted via uridine monophosphate (UMP) pyrophosphorylase into 5-FUMP and 5-FUTP through a 5-FU diphosphate (FUDD) intermediate. 5-FU triphosphate (FUTP) is incorporated into RNA. 5-FU can also be metabolized into F-dUMP, an inhibitor of thymidylate synthetase. A minor pathway converts 5-FU initially into 5,6-dihydrofluorouracil, and then through the intermediates  $\alpha$ -fluoro- $\beta$ -ureidopropionic acid and  $\alpha$ -fluoro- $\beta$ -alanine into fluoride ion and alanine. The information on mechanisms of action and metabolism is compiled from studies by Polak et al. [15, 16], Malet-Martino et al. [17], and Vialaneix et al. [18].

More recently, investigators have begun to use flucytosine successfully in combination with ketoconazole, fluconazole, and itraconazole [40–42]. In an in vitro series compiled by Mary and colleagues [40], flucytosine and ketoconazole were synergistic in 22 of 57 yeast isolates resistant to flucytosine alone, 50 of which were *Candida* species. Allendoerfer et al. [41], using a nude-mouse model of cryptococcal meningitis, showed that the combination of flucytosine and fluconazole prolonged mouse survival and diminished cfu of *C. neoformans* in brain tissue significantly more than did either drug alone. This combination of flucytosine and fluconazole is a potentially exciting alternative to standard therapy for cryptococcal meningitis, since both orally administered agents achieve high concentrations in CSF. Polak [42] noted synergy with the combination of flucytosine and itraconazole in murine candidiasis, particularly against strains resistant to flucytosine alone and also described synergistic and additive effects of flucytosine plus itraconazole in >50% of mice in a disseminated model of aspergillosis.

### Clinical Antifungal Activity

**Cryptococcosis.** A long and detailed compendium of the medical literature regarding the clinical efficacy of flucytosine in the therapy for deep mycoses has been compiled. Most of these studies are single-arm analyses or case reports in which flucytosine was used with or without amphotericin B. Activity of flucytosine as a single agent has been noted in infections produced by *C. neoformans*, *Candida* species, and *T. glabrata*, as well as in chromoblastomycosis and phaeohyphomycosis. However, primary and secondary resistance also were described when flucytosine was used alone.

Early series described cures with the use of flucytosine alone in patients with cryptococcal meningitis refractory to previous therapy with amphotericin B alone. In retrospect, these results likely reflect persistent activity of amphotericin B, given the known long half-life of this agent in tissues [43, 44]. Utz et al. [3] reported clinical improvement and negative CSF cultures in 5 of 11 patients, and in a series by Block and Bennett [45], flucytosine alone produced cures in only 7 of 21 patients with cryptococcal meningitis. In the majority of cases, treatment of cryptococcal meningitis with flucytosine alone is inadequate, given the development of secondary resistance. Amphotericin B alone has a corresponding cure rate of 61%–67% [46]. In view of the known synergy between amphotericin B and flucytosine against *C. neoformans* in vitro, the need for prolonged administration of amphotericin B, and its attendant high cost and toxicity, several investigators have debated the beneficial effects of the combination of the two agents as compared with monotherapy with amphotericin B or, more recently, with fluconazole. The following represents a compilation of the most significant studies relating to this question.

The superiority of the combination of amphotericin B and

flucytosine in cryptococcal meningitis over either drug alone, particularly with regard to sterilization of the CSF, has been borne out in case series [7, 47] and in randomized trials [48, 49], and the combination is now the standard induction regimen in therapy for cryptococcal meningitis [50, 51]. In the study by Bennett and colleagues [49], the combination regimen, given for 6 weeks, resulted in a higher percentage of cure or improvement, a significantly more rapid sterilization of CSF, and improved mortality rates than the 10-week regimen with amphotericin B alone. In addition, the use of flucytosine, by virtue of its synergy with amphotericin B against *C. neoformans*, made a reduction in the dosage of amphotericin B possible and therefore conferred a reduction in nephrotoxicity (an “amphotericin B sparing effect”).

For most patients, a 6-week period of induction therapy is preferred. In a study by Dismukes and the NIH Mycoses Study Group [52], a 4-week induction regimen was advocated in patients with cryptococcal meningitis who had the following features: absence of neurological complications, underlying disease or immunosuppressive therapy; a pretreatment CSF leukocyte count >20/mm<sup>3</sup>; a serum titer of cryptococcal antigen <1:32; and at 4 weeks, negative findings in the CSF India ink preparation and a titer of cryptococcal antigen in serum and CSF <1:8. In that study, however, the relapse rate among patients treated with the 4-week regimen was 11% higher than that among the group treated with the 6-week regimen and the incidence of toxic effects was similar. Given the increasing role of AIDS and immunosuppressive therapy as the underlying factors in most cases of cryptococcal meningitis, the high organism load in patients with AIDS, and the already imperfect role of antifungal therapy for this disease, the emphasis should be placed on improvement in cure rates concomitant with reduction in toxicity.

Chuck and Sande retrospectively reviewed the course and outcome of 89 evaluable patients with AIDS-related cryptococcosis (predominantly meningitis) [53]. Forty-nine patients were treated with the combination of amphotericin B and flucytosine and 40, with amphotericin B alone. Although the survival rate among patients treated with amphotericin B and flucytosine was not significantly better than that among patients treated with amphotericin B alone in their series, there was a trend toward improved survival in those treated with the combination. However, the efficacy of clearance of cryptococcal antigen from the CSF in the two groups was not compared. Moreover, the lack of monitoring of serum concentrations of flucytosine precluded accurate assessment of its toxicity and made interpretation of the role of flucytosine in this series difficult.

In a recent trial by Larsen et al. [54], the combination of amphotericin B and flucytosine demonstrated mycological and clinical efficacy superior to that of fluconazole in AIDS-associated cryptococcal meningitis, despite an increased incidence of adverse effects. Studies of the use of a combination

of amphotericin B, flucytosine, and fluconazole are now under way in an attempt to increase the rate of response to induction therapy [55]. Jones and colleagues recently demonstrated the safety and efficacy of flucytosine and fluconazole in preventing the evolution of cryptococcal meningitis in nine patients positive for human immunodeficiency virus (HIV) with nonmeningeal cryptococcosis [56]. Moreover, preclinical testing by Allendoerfer and colleagues is currently addressing the possible utility of flucytosine and fluconazole as primary therapy for cryptococcal meningitis in an effort to avoid the attendant toxicity of amphotericin B [41].

**Candidiasis.** In older studies, monotherapy with flucytosine demonstrated activity in cases of disseminated candidiasis [2, 57, 58]. For example, flucytosine was curative in 10 of 11 nongranulocytopenic patients with persistent catheter-associated candidemia [59]. Flucytosine has also been used safely in neonates and premature infants, who tolerate amphotericin B poorly [60]. The propensity for emergence of resistance to flucytosine by *Candida* species, however, precludes the use of this agent alone.

More recent experience indicates that candidiasis involving deep tissues, particularly in granulocytopenic patients, is best treated with a combination of amphotericin B and flucytosine. Chronic infections such as candidal endophthalmitis [61–63] and endocarditis [64] are much more refractory to single-agent flucytosine therapy and are best treated with a combination of amphotericin B and flucytosine for prolonged periods along with surgery. There are reports of cure in patients with hepatosplenic candidiasis [65–68] and candidal meningitis [69] treated with the combination of amphotericin B and flucytosine. For treatment of candidal peritonitis, Tapson et al. [70] recommend the combination of amphotericin B and flucytosine along with expedient removal of any catheter. Despite these favorable results, no randomized trials have been performed to clearly determine the superiority of combination antifungal therapy over therapy with amphotericin B alone in the treatment of invasive candidiasis. The combination should be used preferentially in the following conditions of invasive candidiasis [71], especially when they are caused by *C. tropicalis*, *Candida parapsilosis*, *Candida krusei*, or *C. guilliermondii* (which are inherently less susceptible than *C. albicans* to amphotericin B [72–74]: candidal meningitis; hepatosplenic candidiasis; and candidal endophthalmitis, endocarditis, and peritonitis.

**Aspergillosis.** Although susceptibility of *Aspergillus* species to flucytosine in vitro is infrequent, the drug has shown activity in selected cases of aspergillosis [75]. Flucytosine has been used primarily in combination with amphotericin B in cases of invasive aspergillosis refractory to amphotericin B alone [76–78]. In view of the high prevalence of acquired resistance of *Aspergillus* species to flucytosine, along with in vivo evidence of additive effects of flucytosine with amphotericin B, combination therapy should be employed in refrac-

tory cases of pulmonary and disseminated aspergillosis, although the role of high-dose amphotericin B alone vs. the combination is unclear.

**Chromoblastomycosis.** The treatment of chromoblastomycosis caused by dematiaceous molds has been hampered by the relative paucity of effective agents and particularly by the in vitro resistance of many of these molds to amphotericin B. In a small series in the United States [57] and in larger series from Brazil [79, 80], flucytosine has shown impressive activity as sole therapy for chromoblastomycosis. Flucytosine has been administered orally as well as topically outside the United States [81], with an overall cure rate of 72% [58]. Secondary resistance is infrequent but is correlated with the size of the lesion and the duration of therapy.

**Phaeohiphomycosis.** Flucytosine has been used in preclinical studies against the dematiaceous molds responsible for phaeohiphomycosis. Block et al. [82], in a murine model of cladosporiosis, were able to demonstrate a dose-dependent survival rate of 40%–100%, depending on the particular strain used. The 50% effective dose (ED<sub>50</sub>) of flucytosine in these studies was 200–400 mg/(kg·day) for two of the strains, 400–800 mg/(kg·day) for the third, and indeterminate for the fourth. Dixon and Polak [83] demonstrated significant clinical activity of flucytosine as a single agent in mice with cerebral phaeohiphomycosis produced by *Cladosporium bantianum*, *Wangiella dermatitidis*, and *Dactylaria constricta*. Despite exhibiting inhibitory activity only against *C. bantianum* (MIC of 3 µg/mL), flucytosine demonstrated excellent clinical activity against all three pathogens. With a 20-day survival period used as a therapeutic end point, 90% of animals treated with flucytosine at a dosage of 200 mg/(kg·day) survived cerebral infection due to *Wangiella*, 70% treated with 100 mg/(kg·day) survived dactylariosis, and 50% treated with 400 mg/(kg·day) survived cladosporiosis. These results are encouraging, particularly in view of the increased frequency of phaeohiphomycosis in immunocompromised patients [84].

**Other mycoses.** Flucytosine is ineffective in the therapy for blastomycosis, coccidioidomycosis, histoplasmosis, or sporotrichosis.

### Pharmacokinetics

The pharmacokinetics of flucytosine have been reviewed extensively elsewhere [85]. More than 90% of an oral dose is absorbed. In patients with normal renal function, peak levels are attained in serum and other body fluids, including CSF, within 1 to 2 hours [86]. Absorption of flucytosine can be delayed by food, antacids, and renal insufficiency. Flucytosine is poorly bound by plasma proteins, and the volume of distribution approaches that of total body water. Flucytosine penetrates CSF, vitreous, peritoneal fluid [87], inflamed joints [88], and other fluid compartments well. Serum levels of flucytosine are clearly dose-related [89]. Excretion of flu-

cytosine is predominantly (80%–95%) renal by filtration at the glomerulus, without tubular reabsorption or secretion, and is linearly correlated with creatinine clearance [90] according to the following formula:  $t_{1/2} \text{ (h)} = (5.2 \times \text{plasma creatinine [mg/dL]}) + 0.4$ .

In patients with normal renal function the serum half-life ( $t_{1/2}$ ) is 3–4 hours, but in patients with renal insufficiency, it can be several days. Flucytosine is more efficiently eliminated by hemodialysis than by peritoneal dialysis [91], and guidelines based on creatinine clearance have been suggested for administration of flucytosine to patients with renal compromise [85]. In patients with a creatinine clearance of >40 mL/minute, a standard dose of 37.5 mg/kg every 6 hours should be used. If the creatinine clearance is between 20 and 40 mL/minute, the recommended dose is 37.5 mg/kg every 12 hours. In patients with a creatinine clearance of <20 mL/minute, the dose of flucytosine should be 37.5 mg/kg once daily, and if clearance is <10 mL/minute, frequent determinations of serum levels of flucytosine should guide the frequency of dosing. In patients undergoing hemodialysis, a dose of 37.5 mg/kg should be given following dialysis. Ittel and colleagues [92] have described  $t_{1/2}$  values of flucytosine between 15.9 and 37.2 hours in a series of patients with acute renal failure undergoing continuous hemofiltration (CH). They noted an inverse correlation between the filtration rate of CH and the  $t_{1/2}$  and proposed a dosage schedule of flucytosine in patients undergoing CH at various filtration rates [92].

### Toxicity of Flucytosine

Flucytosine has been associated with fatal cases of bone marrow aplasia [4, 5], hepatic necrosis [6], and enterocolitis [7, 8]. Few series have prospectively determined serum levels of flucytosine and correlated them with the development of potentially fatal toxicity (table 3). In a small series by Record et al. [64], levels of flucytosine were >100  $\mu\text{g/mL}$  for a prolonged period in both fatal cases. This series [64], our study, and a multicenter study of antifungal toxicity in the treatment of cryptococcal meningitis conducted by the Mycoses Study Group [52, 93] found that isolated flucytosine levels of  $\geq 100 \mu\text{g/mL}$  that were quickly corrected by a reduction in dosage were not clearly associated with the development of toxicity. For example, the Mycoses Study Group [52, 93] noted flucytosine toxicity (granulocytopenia, thrombocytopenia, and anemia either singly or in combination; elevated hepatic enzyme levels; or gastrointestinal symptoms) in 23 of 38 patients with serum flucytosine concentrations  $\geq 100 \mu\text{g/mL}$  for  $\geq 2$  weeks and in 15 of 48 patients whose flucytosine levels were maintained at <100  $\mu\text{g/mL}$  ( $P = .004$ ) (tables 3 and 4).

Several other investigators [48, 49, 59] (table 3) have reported smaller case series of flucytosine toxicity, in which toxicity was seen when flucytosine levels were >100  $\mu\text{g/mL}$ .

In a review by Kauffman and Frame, four of 15 patients experienced bone marrow toxicity; all four had flucytosine levels >125  $\mu\text{g/mL}$  [9] (table 3). Overall, leukopenia and thrombocytopenia have been reported in 6% of patients; gastrointestinal intolerance (mostly nausea and diarrhea) in 6%; and hepatotoxicity with elevated alkaline phosphatase and transaminase levels in 5% [94].

Myelosuppression and hepatotoxicity in most patients appear to be concentration-dependent, predictable, possibly avoidable with careful maintenance of flucytosine levels at <100  $\mu\text{g/mL}$ , and reversible with temporary discontinuation of the drug or a reduction in dosage. For example, Stamm et al. [93] reported that bone marrow dyscrasias (granulocytopenia and/or thrombocytopenia) occurred in 12 of 20 patients with flucytosine levels  $\geq 100 \mu\text{g/mL}$  as compared with 8 of 65 with flucytosine levels <100  $\mu\text{g/mL}$  ( $P \leq .02$ ); hepatotoxicity developed in 6 of 7 patients with flucytosine levels  $\geq 100 \mu\text{g/mL}$  as compared with 1 of 78 with flucytosine levels <100  $\mu\text{g/mL}$  ( $P \leq .004$ ). By comparison, the preponderance of patients reported in this current study had mean flucytosine levels <100  $\mu\text{g/mL}$  or serum levels only transiently elevated to  $\geq 100 \mu\text{g/mL}$ . These patients experienced minimal toxicity attributable to flucytosine.

As the findings of Stamm et al. [93] and our own data indicate, the relationships between flucytosine-induced bone marrow suppression and hepatotoxicity are general and not absolute. For example, some series, including our own, have described patients who suffered adverse effects such as hepatotoxicity or eosinophilia that were idiosyncratic and not related to the flucytosine level [47, 49, 52, 59, 93] (tables 2 and 4). Other patient populations (e.g., HIV-infected patients) may experience thrombocytopenia or leukopenia that is not associated with elevated serum levels of flucytosine, findings suggesting a greater sensitivity to concentration-dependent myelosuppression. Conversely, not all patients with clearly elevated flucytosine levels will experience adverse effects [9, 48, 49, 52, 93, 95] (tables 2 and 5). The factors responsible for this phenomenon are as yet unknown. Skin rash [94], eosinophilia [59, 96], and crystalluria [97] are less common adverse effects than myelosuppression and hepatotoxicity.

According to the 1992 edition of the *Physicians' Desk Reference* (PDR), the advised dosage range for flucytosine is 50–150 mg/kg daily in four divided doses [98]. We have found that administration of flucytosine at a dosage of 150 mg/(kg·day) in combination with amphotericin B at 0.5–1.0 mg/(kg·day) in patients with initially normal serum creatinine values often results in elevated serum levels of flucytosine ( $\geq 100 \mu\text{g/mL}$ ) that necessitate a reduction in dosage within several days of initiation of therapy. We suggest that adjustment of the higher doses of flucytosine be guided by results of serial determinations of flucytosine levels to minimize toxicity.

**Table 3.** Toxicities of flucytosine (5-FC) associated with elevated serum levels reported in the literature.

Reference, patient no.	Renal dysfunction	Serum 5-FC levels (µg/mL)	Toxicities
Record et al. [64]			
1	Yes	100-180	Patchy hepatic necrosis, pancytopenia, and death
2	Yes	110-130	Anemia, patchy hepatic necrosis, and death
Eilard et al. [59]			
3	Not stated	100-150	Nausea
4	Not stated	100-150	Eosinophilia
Utz et al. [48]			
5	Yes	66-155	Anemia and hepatotoxicity
6	No	29-150	Thrombocytopenia and leukopenia
7	No	60-150	Anemia, anorexia, nausea, and vomiting
Kauffman and Frame [9]			
8	Yes	500 (peak)	Pancytopenia and death
9	Yes	200 (peak)	Leukopenia
10	No	125 (peak)	Leukopenia
11	Yes	160 (peak)	Leukopenia
Bennett et al. [49]			
12	Yes	>100	Leukopenia and thrombocytopenia
13	Yes	>100	Thrombocytopenia
14	Yes	>100	Diarrhea
15	Yes	>100	Leukopenia
16	Yes	>100	Leukopenia
17	Yes	>100	Rash, leukopenia, and diarrhea
Mycoses Study Group [52], 18-55	Not stated	>100	6/7 with hepatotoxicity, 12/20 with blood dyscrasias, 5/11 with gastrointestinal symptoms

**Miscellaneous Factors Influencing Toxicity of Flucytosine**

Although their influence is controversial, two factors, amphotericin B-induced renal insufficiency and secondary metabolites of flucytosine and gastrointestinal flora, may influence flucytosine toxicity.

**Table 4.** Toxicities of flucytosine (5-FC) not associated with elevated serum levels reported in the literature.

Reference, patient no.	Serum 5-FC level (µg/mL)	Toxicities
Eilard et al. [64]		
1	30-70	Hepatotoxicity
2	30-70	Eosinophilia
3	30-70	Eosinophilia
4	30-70	Eosinophilia
Utz et al. [48]		
5	48-96	Leukopenia
6	87	Thrombocytopenia
Bennett et al. [49], 7	<100	Leukopenia
Mycoses Study Group [52], 8-22	<100	Not specified

*Amphotericin B-induced renal insufficiency.* Most patients with elevated flucytosine levels have concomitant nephrotoxicity in conjunction with concurrent or previous treatment with amphotericin B, a factor known to markedly prolong the duration of serum t<sub>1/2</sub> of flucytosine to >24 hours [85, 86]. This effect is exemplified in a recent study of leukemic patients [99], in whom granulocyte recovery was prolonged with empirical antifungal prophylaxis consisting of a combination of amphotericin B and flucytosine as compared with recovery in a similar population that did not re-

**Table 5.** Elevated serum levels of flucytosine (5-FC) not associated with toxicity reported in the literature.

Reference	Patient no.	Serum 5-FC levels (µg/mL)
Steer et al. [95]	1	44-150
	2	122-140
Utz et al. [48]	3	113-180
Kauffman and Frame [9]	4	125
Bennett et al. [49]	5-9	>100
Mycoses Study Group [52]	10-23	>100

ceive such prophylaxis. In that study, only five of 22 patients who received the combination actually had proven fungal infection. Renal dysfunction in the two groups was not mentioned, and measurement of flucytosine levels not performed. Rather than using flucytosine empirically, we add flucytosine to amphotericin B in the setting of proven invasive fungal infection.

The mechanism of dose-dependent flucytosine toxicity may be related directly to the conversion of flucytosine to fluorouracil. For example, Kissling et al. [100] found that concentrations of flucytosine of  $\geq 100 \mu\text{g/mL}$  alone or in combination with amphotericin B inhibited burst/colony formation of human bone marrow precursor cells *in vitro* but fluorouracil levels had a more suppressive effect.

Further illustrating the potential contributory role of nephrotoxic agents in the toxicity of flucytosine is the observation that, when flucytosine was administered chronically as a single agent to patients in South America [79] with chromoblastomycosis, adverse effects were uncommon, even when doses were  $200 \text{ mg}/(\text{kg} \cdot \text{day})$ . Moreover, early studies employing flucytosine as a single agent in the treatment of invasive candidiasis, cryptococcosis, and fungal endocarditis demonstrated activity with little toxicity [57, 59, 64]. However, measurements of flucytosine levels are lacking in these reports.

*Secondary metabolites of flucytosine and gastrointestinal flora.* Recently Malet-Martino et al. [17] and Vialaneix and colleagues [18], using  $^{19}\text{F}$  nuclear magnetic resonance imaging, confirmed the existence of several detectable metabolites of flucytosine in addition to fluorouracil in bodily fluids, albeit in small quantities. These metabolites include 5,6-dihydrofluorouracil,  $\alpha$ -fluoro- $\beta$ -ureidopropionic acid [16],  $\alpha$ -fluoro- $\beta$ -alanine, fluoride ion, 6-hydroxy-5-fluorocytosine, and 5-fluorocytosine glucuronide (figure 2). The extent to which these metabolites produce toxicity is still unclear. In addition, broad-spectrum antibiotics, which alter gastrointestinal flora, may reduce the expression of some of these metabolites [17].

## Discussion

In view of these findings, we reviewed the clinical courses of 17 evaluable patients who had been treated with the combination of amphotericin B and flucytosine for deep mycoses. In 10 of 17 patients, the combination of amphotericin B and flucytosine successfully eradicated the mycosis with concomitant patient survival. The addition of flucytosine to amphotericin B eradicated the fungal infection in six of 10 patients whose infection developed or progressed during treatment with amphotericin B alone. The combination regimen failed in only three of 10 patients with candidemia, results representing an improvement over the mortality rates of  $\sim 80\%$  in patients with cancer treated for fungemia with other regimens [101].

Determination of serum levels of flucytosine was critical to the management of invasive mycoses in these immunosuppressed patients and broadened the therapeutic window of a potentially toxic compound. The vast majority of serum flucytosine levels were maintained at  $< 100 \mu\text{g/mL}$ . Levels exceeding this value were quickly and appropriately corrected by temporarily discontinuing the drug or reducing the dosage. For this reason, the major toxicities reported in earlier literature were averted in this series and the adverse effects encountered herein were easily managed.

Peak serum levels of flucytosine were not found to be significantly greater than trough levels at steady-state in patients treated with the oral preparation (figure 1). A 30-minute interval between administration of flucytosine and the withdrawal of a serum sample could explain this observation, presumably because of the more rapid availability of the intravenous preparation. These findings suggest that determining a serum level for orally administered flucytosine 30–60 minutes after a dose may obviate the need for determining a level before a dose, especially during the steady state. In comparison to the different peak and trough levels observed with intravenous administration of flucytosine, the approximation of the times to reach peak and trough levels of the orally administered compound does not appear to have been widely appreciated. A peak level occurring 2 hours after dosing might reveal a delayed absorption, but the differences are unlikely to be significant.

As a further marker of the safety of flucytosine when dosage is guided by serum levels, flucytosine did not produce granulocytopenia in any of the eight patients who were not granulocytopenic.

Hepatotoxicity with elevated AST, ALT, alkaline phosphatase, and total bilirubin levels was possibly attributable to flucytosine in only one patient (table 2). However, mean levels in this patient were  $34 \mu\text{g/mL}$  before and  $60.8 \mu\text{g/mL}$  after the flucytosine dose (table 1). Moreover, after administration of flucytosine was temporarily discontinued, the patient tolerated reinstitution of the same dose and experienced complete resolution of hepatosplenic candidiasis without progressive liver dysfunction. Factors such as the use of broad-spectrum antibiotics, myeloablative regimens for bone-marrow transplantation, viruses, residual tumor, and multiple transfusions in this patient, as well as in other immunocompromised patients with invasive mycoses, may also have contributed to hepatotoxicity.

Gastrointestinal symptoms attributable to flucytosine were transient and manifested by nausea and diarrhea in one patient each. The nausea was associated with mean levels of flucytosine of  $90.2 \mu\text{g/mL}$  before and  $96.2 \mu\text{g/mL}$  after the dose, suggesting a possible concentration-dependent adverse effect. By comparison, the patient with diarrhea was noted to have mean flucytosine levels of  $50.3 \mu\text{g/mL}$  before and  $53 \mu\text{g/mL}$  after the dose, suggesting little or no relation between concentrations and adverse effect.

Since flucytosine usually is administered with amphotericin B and the adverse effects of 5-FC are primarily dose-related, it has become necessary to measure flucytosine levels, especially in patients with renal insufficiency. A variety of methods for doing this have been proposed, with relative differences in requisite expertise, cost, accuracy, turnaround time, and reproducibility. The methods include bioassay [102, 103], gas-liquid chromatography (GLC) [104, 105], fluorimetry [106, 107], high-performance liquid chromatography (HPLC) [108–112], and, more recently, enzymatic methods [10–14].

The bioassay and its several modifications correlate the sizes of zone inhibition around filter disks or wells impregnated with known concentrations of flucytosine and the test serum with the serum concentration of flucytosine. Minimal technical expertise and apparatus are required, permitting widespread use of this method at low cost. However, the bioassay lacks the precision and rapidity necessary for making efficient dose adjustments, and its reproducibility has also been questioned.

GLC methods, which require derivatization of flucytosine, and fluorimetric methods have largely been abandoned in favor of HPLC assays using ion-exchange, ion-pair, and reversed-phase columns. These are rapid (<30 minutes), specific, and accurate, permitting dose adjustments in patients with azotemia. The main drawbacks are the cost and technical expertise required.

On the other hand, the creatinine iminohydrolase assay takes advantage of a fortuitous observation that flucytosine, by virtue of a close structural resemblance to creatinine, causes spurious creatinine elevation in sera as measured by the Kodak Ektachem analyzer (Eastman Kodak, Rochester, NY) [113–117]. Such apparatus is widely available; no additional technical expertise or cost is required; and the results are precise, expedient, and correlate well with results of HPLC methods [10, 14]. As a result, this assay is particularly attractive for making serial measurements to monitor drug-induced toxicity. In the creatinine iminohydrolase assay as initially described [11, 12],  $\text{NH}_3$  diffused through a semipermeable membrane and reacted with a bromphenol blue indicator. In the assay used in this report, the liberation of  $\text{NH}_3$  is measured as the difference between the spectrophotometric absorbance of the serum sample and a blank control. The creatinine iminohydrolase assay may be positively influenced by the presence of lipids, therefore chylomicron-free serum should be used [10]. Elevated bilirubin concentrations may affect measured flucytosine concentrations positively by a maximum of 10%, but the assay is not affected by the presence of other pyrimidines, amphotericin B, glucose, or elevated creatinine concentrations.

In this series, the results of assays for flucytosine levels were rapidly available and allowed adjustment of the dose. Only two patients (nos. 2 and 17) had mean flucytosine levels in the toxic range, albeit briefly. Only one patient (no. 12)

demonstrated borderline high levels of flucytosine in the setting of possible gastrointestinal toxicity. In the opinion of the physicians caring for these patients, none clearly exhibited granulocytopenia or thrombocytopenia related to flucytosine. One patient (no. 10) had hepatic dysfunction that correlated temporally with the administration of flucytosine; however, serum levels were below the usual toxic range. Finally, no patients in this closely monitored setting experienced any severe reactions such as marrow aplasia, hepatocellular necrosis, or enterocolitis attributable to flucytosine.

No definitive conclusions can be drawn from these data regarding the efficacy of flucytosine in the treatment of these patients because it was used concomitantly with amphotericin B in a nonrandomized setting. In fact, to date no randomized trials have been reported in the literature that compare the efficacy of amphotericin B with the combination of amphotericin B and flucytosine in the treatment of disseminated candidiasis. Nonetheless, flucytosine clearly demonstrated antifungal activity in six of 10 patients in this series whose infection developed or progressed during treatment with amphotericin B alone.

The optimal dosage of flucytosine has not been rigorously defined. A balance is sought between antifungal activity and host toxicity. During the course of therapy with flucytosine, we routinely adjust the dose of flucytosine to maintain peak serum levels between 40 and 60  $\mu\text{g}/\text{mL}$ . This range of flucytosine levels provides a good margin of safety, particularly in patients with changing renal function and whose serum levels of flucytosine may increase rapidly to concentrations that exceed 100  $\mu\text{g}/\text{mL}$ . The MICs of most susceptible fungi are <10  $\mu\text{g}/\text{mL}$  [24, 33], depending on the methods used to determine in vitro susceptibility. Moreover, Normark and Schönebeck [23] found that levels of flucytosine that were >25  $\mu\text{g}/\text{mL}$  did not contribute further to the prevention of spontaneous emergence of resistance by *C. albicans* and *T. glabrata*. The question of whether lower levels of flucytosine may be used without compromising the therapeutic effect of combination therapy remains to be studied. These lower levels may be particularly beneficial in patients with HIV infection, some of whom may not tolerate conventional dosages of flucytosine. Currently, however, in our patient population the peak serum levels of 40–60  $\mu\text{g}/\text{mL}$  appear to provide a reasonable balance of the factors of antifungal activity, toxicity, and prevention of emergence of resistance.

In summary, the use of antibiotics is associated with adverse effects that must be considered during their administration. Fortunately, levels of selected antimicrobial agents can be expediently measured to decrease the ratio of risk to benefit. For example, the aminoglycosides are extremely useful compounds whose nephrotoxicity and ototoxicity are well recognized yet can potentially be avoided by close measurement of drug levels. Similarly, flucytosine can produce severe adverse effects, particularly granulocytopenia, if administered to azotemic patients without monitoring flucytosine

levels. Hence, flucytosine should be used only in cases of documented fungal infection and not as a part of a program of prophylactic therapy for patients with granulocytopenia and prolonged fever. Furthermore, in granulocytopenic patients with invasive mycoses, flucytosine should be administered only in combination with amphotericin B. A clear indication for the use of the combination is induction therapy for cryptococcal meningitis. In patients with acute disseminated candidiasis; hepatosplenic candidiasis; candidal endophthalmitis, endocarditis, meningitis, or peritonitis; or invasive candidiasis caused by non-*albicans* species, addition of flucytosine to amphotericin B should be strongly considered. If the use of flucytosine is restricted to these situations and if renal function and serum flucytosine levels are closely monitored and the latter are maintained between 40 and 60  $\mu\text{g}/\text{mL}$ , the combination of amphotericin B and flucytosine can be effective and minimally toxic.

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