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Oral versus Intravenous Flucytosine in Patients with Human Immunodeficiency Virus-Associated Cryptococcal Meningitis

Annemarie E. Brouwer,1,2,3 Hendrikus J. M. van Kan,4 Elizabeth Johnson,5 Adul Rajanuwong,6 Prapit Teparrukkul,6 Vannaporn Wuthiekanun,2 Wirongrong Chierakul,2 Nick Day,2,7 and Thomas S. Harrison1*

Centre for Infection, St. George’s University of London, United Kingdom1; Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand2; Department of Internal Medicine and Nijmegen University Centre for Infectious Diseases, University Medical Centre Nijmegen, The Netherlands3; Department of Clinical Pharmacy, Academic Medical Centre, Amsterdam, The Netherlands4; Mycology Reference Laboratory, Health Protection Agency South-West Regional Laboratory, Bristol, United Kingdom5; Department of Medicine, Sappasithiprasong Hospital, Ubon Ratchathani, Thailand6; and Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom7

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In a randomized controlled trial of amphotericin B-based therapy for human immunodeficiency virus (HIV)-associated cryptococcal meningitis in Thailand, we also compared the mycological efficacy, toxicity, and pharmacokinetics of oral versus intravenous flucytosine at 100 mg/kg of body weight/day for the initial 2 weeks. Half of 32 patients assigned to the two arms containing flucytosine were randomized to oral and half to intravenous flucytosine. Early fungicidal activity was determined from serial quantitative cultures of cerebrospinal fluid (CSF), and toxicity was assessed by clinical and laboratory monitoring. Flucytosine and fluorouracil concentrations in plasma and CSF were measured by high-performance liquid chromatography. No significant bone marrow or hepatotoxicity was seen, there was no detectable difference in bone marrow toxicity between patients on intravenous and those on oral formulation, and no patients discontinued treatment. In patients receiving intravenous flucytosine, the median 24-h area under the concentration-time curve was significantly higher than in the oral group. Despite this difference, there was no difference in early fungicidal activity between patients on intravenous compared with patients on oral flucytosine. The results suggest that either formulation can be used safely at this dosage in a developing country setting, without drug concentration monitoring. The bioavailability of the oral formulation may be reduced in late-stage HIV-infected patients in Thailand. Concentrations of flucytosine with intravenous formulation at 100 mg/kg/day may be in excess of those required for maximal fungicidal activity.

Flucytosine (5FC) in combination with amphotericin B (AMB) is standard therapy for cryptococcal meningitis in the United States and Europe. 5FC is taken up by fungal cells by cytosine permease and converted into fluorouracil (5FU) by fungal cytosine deaminase. Further metabolism of 5FU leads to the formation of 5-fluorouridine triphosphate, which is incorporated into fungal RNA, and 5-fluorodeoxyuridine monophosphate, an inhibitor of thymidylate synthetase. This results in inhibition of protein and DNA synthesis in the fungal cell (19).

Side effects of 5FC include nausea, vomiting, diarrhea, bone marrow depression, and hepatotoxicity. The latter two are thought to be due to effects of 5FU. Human cells lack the enzyme cytosine deaminase and are unable to convert 5FC into 5FU. However, the human intestinal microflora has been shown to be capable of converting 5FC into 5FU in vitro (8, 10, 17), and 5FU, at concentrations known to be associated with bone marrow depression, has been measured in the plasma of patients treated with oral 5FC (6). If intestinal bacteria do play a role in conversion of 5FC to 5FU in patients, then oral administration of 5FC might be associated with increased 5FU concentrations and more side effects than intravenous (i.v.) administration of the drug. On the other hand, i.v. 5FC is more costly to administer in resource-poor settings and carries the added inconvenience of strict storage temperature requirements. Therefore, in the context of a trial of combination antifungal therapy for human immunodeficiency virus (HIV)-associated cryptococcal meningitis, we compared the efficacy, toxicity, and pharmacokinetics of oral versus i.v. 5FC.

MATERIALS AND METHODS

Participants and procedures. The study was approved by the ethical and scientific review subcommittee of the Thai Ministry of Public Health and by the research ethics committee of St. George’s University of London and was carried out at Sappasithiprasong Hospital, Thailand, as described previously (4). With written informed consent, we enrolled 64 adults with a first episode of cryptococcal meningitis, diagnosed by cerebrospinal fluid (CSF) India ink and cryptococcal antigen tests. Exclusion criteria were an alanine aminotransferase concentration of more than five times the upper limit of normal, a neutrophil count of less than 0.5 x 10^9/liter, a platelet count of less than 50 x 10^9/liter, pregnancy, and previous serious reaction to study drugs. The participants were randomized to give equal numbers in each of four treatment arms: amphotericin B deoxycholate alone (0.7 mg/kg of body weight daily, Fungizone; Bristol-Myers Squibb, New York, NY); amphotericin B plus fluconazole (400 mg daily, Diflucan; Pfizer, New York, NY); amphotericin B plus flucytosine (100 mg/kg daily, Ancotil [i.v. formulation; Valeant, Zoetermeer, The Netherlands].

* Corresponding author. Mailing address: Centre of Infection, St George’s University of London, London SW17 ORE, United Kingdom. Phone: 44 20 8725 0447. Fax: 44 20 8725 3487. E-mail: tharriso@sgu.ac.uk.

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and Ancobon [oral tablets; Basingstoke, United Kingdom]); and triple therapy
with amphotericin B, flucytosine, and fluconazole. Half of the patients in each of
the two arms containing 5FC were randomized to oral and half to i.v. 5FC.
Treatment was not blinded. Unless contraindicated, patients received 1 liter of
0.9% (normal) saline daily to keep AMP nephrotoxicity to a minimum. After 2
weeks, we treated all four arms with fluconazole, 400 mg daily for 8 weeks, and
200 mg daily thereafter (Fuzoral; Government Pharmaceutical Organization,
Thailand).

**Efficacy.** The rate of clearance of infection, or early fungicidal activity, of oral
versus i.v. flucytosine was determined from quantitative cultures of CSF at
baseline and days 3, 7, and 13 or 14 of treatment, using the slope of the linear
regression of log CFU against time for each patient, as previously described (4).
Negative CSF cultures were assigned a value of 1 CFU per ml. All data points
were used, with the exception of sterile cultures at 14 days if this value reduced
the slope. In these cases, CSF sterility would likely have been achieved at some
time between 7 and 14 days, and use of the 14-day result would therefore lead to
an underestimation of the true slope (4).

**Clinical and laboratory toxicity.** Patients were monitored daily during the
initial 2 weeks of therapy for the development of clinical side effects. Peripheral
blood samples were taken at admission and on days 3, 5, 7, 9, 11, and 13 for
test and electrolytes and at admission and on days 5, 9, and 13 for full
blood count and liver function tests. Absolute values at day 13 and percent
change at day 13 compared to baseline were taken as laboratory outcomes. If
there was no day 13 value, the latest available follow-up value was used.

**Pharmacokinetics.** Blood for 5FC concentrations was taken on days 3 and 9
and on an average of three other days. Times of administration of 5FC and of the
vena puncture were registered at the bedside of the patient. A mean of 5 samples
per patient were frozen at –20°C, and 5FC and SFU concentrations were
determined simultaneously using reversed-phase high-performance liquid chroma-
tography with UV detection at the Amsterdam Medical Center after completion
of the study (15). 5FC concentrations in CSF were determined at day 14 with a
similar method at the Mycology Reference Laboratory, Bristol, United Kingdom.
Both methods had between-run variation coefficients below 6% within the
quantification range. Individual pharmacokinetic parameters were calculated using
a Bayesian maximum a posteriori fitting procedure using the computer program
MW/Pharm version 3.60 (Mediware, The Netherlands) (12, 18). The Bayesian
fitting procedure uses measured drug concentrations and population-based phar-
macokinetic parameters to determine individualized pharmacokinetic parameters
of a patient (12). We used a one-compartment open model. The initial
pharmacokinetic parameters of 5FC were taken from a previous study (18):
elimination rate constant normalized to creatinine clearance ($k_e/\text{Cr}$), 0.0009 ±
0.0005 min/ml -h; volume of distribution normalized to weight ($V_d/\text{kg}$), 0.899 ±
0.43 liters/kg; and oral absorption rate constant ($k_a$), 2 h$^{-1}$.

**MIC.** The MICs of 5FC for the isolates were determined at the Mycology
Reference Laboratory, Bristol, United Kingdom, by means of the CLSI (for-
merly NCCLS) method M27–A2 (11). This method was modified as suggested in
the document by the use of yeast nitrogen base as the basal medium to improve
the clinical relevance of the antifungal MIC. Briefly, broth microdilution was
performed in 96-well round-bottom microtiter plates containing 100 μl of dou-
bbling dilutions of 5FC to which were added 100-μl volumes of an inoculum
suspending of 0.5 × 10$^4$ to 2.5 × 10$^4$ cells/ml in yeast nitrogen base. Plates were
incubated at 35°C and read after 72 h, with an 80% growth inhibition defined as
the end point.

**Statistics.** Baseline characteristics of groups were compared by the chi-square
test for categorical variables and the Mann-Whitney U test or Student’s t test for
continuous variables. The Mann-Whitney U test and Wilcoxon matched pairs
test were used for comparing laboratory outcomes. Given a comparison of five
laboratory variables, a P value of 0.01 was considered significant (Bonferroni
adjustment for multiple comparisons). Fisher’s exact test and Mann-Whitney U
test were used to compare pharmacokinetic parameters, and linear regression (4,
14) was used to compare fungicidal activity.

**RESULTS**

Sixty-four patients were enrolled. One patient, who was
HIV-seronegative, was excluded. Thirty-one patients were as-
signed to 5FC treatment, with 16 assigned to the oral for-
mulation and 15 assigned to i.v. formulation. The baseline
clinical characteristics of the patients have been previously
reported (4). Baseline laboratory values are shown in Table 1.
Values for patients in the different groups were similar, except

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall (n=63)</th>
<th>5FC (n=31)</th>
<th>No-5FC (n=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (m/f)</td>
<td>38 (69%)</td>
<td>20 (64%)</td>
<td>18 (57%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>47 (9±17)</td>
<td>47 (9±17)</td>
<td>47 (9±17)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74 (60–82)</td>
<td>75 (68–83)</td>
<td>74 (60–82)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 (150–170)</td>
<td>160 (150–170)</td>
<td>160 (150–170)</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25.9 (23.0–30.9)</td>
<td>25.9 (23.0–30.9)</td>
<td>25.9 (23.0–30.9)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>74 (64–82)</td>
<td>74 (64–82)</td>
<td>74 (64–82)</td>
</tr>
<tr>
<td>AST (U/liter)</td>
<td>24 (15–30)</td>
<td>24 (15–30)</td>
<td>24 (15–30)</td>
</tr>
<tr>
<td>ALT (U/liter)</td>
<td>20 (10–30)</td>
<td>20 (10–30)</td>
<td>20 (10–30)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.9 (12.0–13.8)</td>
<td>12.9 (12.0–13.8)</td>
<td>12.9 (12.0–13.8)</td>
</tr>
<tr>
<td>Neutrophils (10$^9$/liter)</td>
<td>7.2 (6.0–8.5)</td>
<td>7.2 (6.0–8.5)</td>
<td>7.2 (6.0–8.5)</td>
</tr>
<tr>
<td>Platelets (10$^9$/liter)</td>
<td>194 (150–250)</td>
<td>194 (150–250)</td>
<td>194 (150–250)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39 (36–43)</td>
<td>39 (36–43)</td>
<td>39 (36–43)</td>
</tr>
<tr>
<td>CSF cryptococcal antigen titer</td>
<td>32 (16–64)</td>
<td>32 (16–64)</td>
<td>32 (16–64)</td>
</tr>
<tr>
<td>CSF cryptococcal culture</td>
<td>102 (51–204)</td>
<td>102 (51–204)</td>
<td>102 (51–204)</td>
</tr>
<tr>
<td>CSF sterile CSF</td>
<td>102 (51–204)</td>
<td>102 (51–204)</td>
<td>102 (51–204)</td>
</tr>
<tr>
<td>CSF albumin (g/liter)</td>
<td>0.3 (0.2–0.4)</td>
<td>0.3 (0.2–0.4)</td>
<td>0.3 (0.2–0.4)</td>
</tr>
<tr>
<td>CSF glucose (mmol/liter)</td>
<td>2.4 (2.0–2.6)</td>
<td>2.4 (2.0–2.6)</td>
<td>2.4 (2.0–2.6)</td>
</tr>
</tbody>
</table>

**Baseline clinical and laboratory characteristics.**
for the fact that the baseline median neutrophil count was lower in patients not receiving 5FC (3.6 × 10^9/liter; interquartile range [IQR], 2.6 to 4.7) compared with those assigned to 5FC (4.7 × 10^9/liter; IQR, 3.6 to 6.1; \( P = 0.008 \), Mann-Whitney U test) (Table 1).

Early fungicidal activity was significantly greater in patients treated with 5FC than in patients not treated with 5FC (−0.46 [standard deviation (SD), 0.18] versus −0.34 [SD, 0.17] log CFU/day, respectively; \( P = 0.02 \), linear regression) (Fig. 1). There was no difference in early fungicidal activity in patients on i.v. compared to patients on oral 5FC (−0.43 [SD, 0.13] versus −0.48 [SD, 0.22] log CFU/day, respectively; \( P = 0.45 \), linear regression). There was still no difference if linear regression was used to adjust for the effect of host immunity (baseline CSF gamma interferon concentrations) (14) on the rate of clearance of infection. There was no difference in mortality comparing patients on 5FC with patients not on 5FC and comparing patients on i.v. 5FC with patients on oral 5FC.

All treatment arms were well tolerated, and no drug treatment had to be withdrawn within the first 2 weeks because of clinical or laboratory side effects. Nine of 63 patients (14%) died during the first week of treatment, and one patient left the hospital on day 2, so that samples from the end of initial therapy were available from 53 (84%) patients, 25 in the non-5FC arms and 28 in the 5FC arms. There was no severe bone marrow depression (neutrophils < 0.5 × 10^9/liter or platelets < 50 × 10^9/liter) and no clinically significant rise in liver function tests (>5-fold above normal).

Taking all patients, hemoglobin levels and calculated creatinine clearance decreased significantly over the initial 2 weeks of treatment (\( P < 0.0001 \), Wilcoxon matched pairs test). The magnitude of the reductions was not affected by treatment with 5FC or mode of administration of 5FC. There was no significant difference in any laboratory parameters, as a percentage of the baseline (Table 2) or in absolute values (data not shown), comparing patients on 5FC with patients not on 5FC and comparing patients on i.v. 5FC with patients on oral 5FC.

5FC concentrations were measured on an average of 5 occasions per patient in 28 patients on 5FC treatment. Pharmacokinetic parameters were calculated. The median oral clearance (CL/F) was 4.54 liters/h (IQR, 3.21 to 6.79 liters/h), the median volume of distribution normalized to weight (\( V_d/kg \)) was 1.16 liters/kg (IQR, 0.74 to 1.67 liters/kg), and the oral absorption rate constant (\( k_a \)) was 1.72 h\(^{-1}\) (IQR, 0.21 to 2.67 h\(^{-1}\)). The median 24-h area under the concentration-time curve (AUC\(_{24}\)) during steady state was significantly higher in patients on i.v. 5FC than in patients on oral 5FC (at day 7, 1,289 [IQR, 721 to 1,637] versus 576 [IQR, 455 to 847] mg · h/liter; \( P = 0.002 \), Mann-Whitney U test) (Table 3). There was no correlation between AUC\(_{24}\) and either absolute values or percent change from the baseline in any of the laboratory variables at the end of 2 weeks of treatment (Spearman’s
correlation, $P > 0.05$). In 5 patients, all on i.v. treatment, peak plasma 5FC concentrations above 100 mg/liter were measured on at least one occasion. No patients on oral 5FC had concentrations above 75 mg/liter at any time. High concentrations were not associated with bone marrow toxicity or hepatotoxicity (results not shown). 5FU was detected in 4 patients (0.50, 0.97, 1.25, and 1.28 mg/liter), 3 on oral 5FC and 1 on i.v. 5FC treatment. There was no association between the presence of 5FU and hemoglobin, neutrophil, or platelet count or ALT or AST (aspartate aminotransferase) at the end of 2 weeks of treatment.

The 5FC MIC ranged from 1 to 8 mg/liter. In patients treated with 5FC, there was no significant correlation of 5FC MIC, area under the concentration-time curve (AUC), AUC/MIC ratio, or time above MIC (which was 100% for all except one patient), with the rate of clearance of infection.

CSF 5FC concentrations at the end of initial therapy were 84% of corresponding plasma concentrations (median IQR, 57 to 106; $n = 19$). Again, no association was found between CSF concentrations and rate of clearance of infection from the CSF.

**DISCUSSION**

In this study, 5FC was safe at 100 mg/kg/day for 2 weeks in patients with cryptococcal meningitis. The results support the safety of 5FC, at this dosage and without drug concentration monitoring, seen in the last Mycoses Study Group trial (16) and extend the findings to a developing country setting. While 5FC remains a part of our most rapidly fungicidal combination for initial therapy, the results argue for more widespread access to 5FC in Southeast Asia and Africa (2), areas where increasing availability of antiretroviral drugs now offers patients with HIV-associated cryptococcal meningitis the prospect of a good long-term prognosis, provided they survive the acute cryptococcal infection.

Despite significantly lower concentrations of 5FC in patients on oral 5FC, 5FU was detected more frequently in patients on oral compared with the i.v. formulation. The data are compatible with the hypothesis that intestinal microflora plays some role in the conversion of 5FC to 5FU. However, at these doses, any conversion of 5FC to 5FU was not associated with detectable toxicity.

CSF concentrations were 84% of corresponding plasma concentrations, in agreement with prior data (3). Plasma concentrations were significantly higher in patients given the i.v. formulation than in patients given the oral formulation. Absorption and bioavailability of oral 5FC has generally been high (75 to 90%) in normal volunteers and patients in North America (3, 5). The data suggest that absorption is not so complete in patients with late-stage HIV disease in Thailand, with the ratio of AUC for oral and i.v. formulations suggesting an oral bioavailability of only 45%.

Despite the difference in 5FC plasma concentrations, there was no difference in mycological efficacy, as accurately assessed by serial quantitative cultures. The results support in vitro and animal model work suggesting that 5FC has concentration-

**TABLE 3. Plasma pharmacokinetic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oral ($n = 14$)</th>
<th>i.v. ($n = 14$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated peak (mg/liter)</td>
<td>30 (23–35)</td>
<td>63 (41–82)</td>
<td>&lt;0.00001&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Calculated trough (mg/liter)</td>
<td>20 (13–28)</td>
<td>37 (17–57)</td>
<td>0.004&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Clearance/F (liter/h)</td>
<td>6.7 (4.8–8.3)</td>
<td>3.8 (2.7–4.3)</td>
<td>0.006&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>$\kappa_{el}$ (min/ml/h)</td>
<td>0.0017 (0.0008–0.022)</td>
<td>0.0014 (0.0009–0.0024)</td>
<td>0.89&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>$V_{d}$/kg (liter/kg)</td>
<td>1.41 (0.85–2.23)</td>
<td>0.94 (0.60–1.18)</td>
<td>0.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5FC AUC&lt;sub&gt;24&lt;/sub&gt; (mg · h/liter)</td>
<td>576 (455–847)</td>
<td>1,289 (721–1,637)</td>
<td>0.002&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5FC &gt; 100 mg/liter&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0</td>
<td>5</td>
<td>0.04&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5FC &gt; 75 mg/liter&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0</td>
<td>8</td>
<td>0.002&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Any 5FU&lt;sup&gt;f&lt;/sup&gt;</td>
<td>3</td>
<td>1</td>
<td>0.58&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Peak and trough concentrations were calculated on days 3, 7, and 13. The AUC<sub>24</sub> was determined on day 7.

<sup>b</sup> Results are median percentages of baseline values with IQR indicated in parentheses.

<sup>c</sup> Results are medians with IQR indicated in parentheses.

<sup>d</sup> Mann-Whitney U test.

<sup>e</sup> Fisher exact test.

<sup>f</sup> All values are the median percentages of baseline values with IQR indicated in parentheses.

<sup>g</sup> CrClear (creatinine clearance) = (140 – age in years) × (weight in kg)/(72 × serum creatinine in mg/dl); for women, multiply by 0.85.
independent pharmacodynamics (1, 7, 9). Plasma concentrations between 20 and 30 mg/liter, as seen in patients on the oral formulation, were not associated with any reduction in fungicidal activity. The facts that maximal fungicidal activity, additional to the effect of AMB, was achieved, and that the time above MIC was close to 100% with this dosage of 5FC, irrespective of mode of administration, may explain why we could not find an association between any pharmacokinetic parameter and rate of clearance of infection and between the narrow range of MICs and rate of clearance of infection. With regard to the MIC, it is of interest that Schwartz and colleagues have shown additive effects with 5FC given with AMB in a murine model, even when the isolate was resistant to 5FC (MIC = 64 μg/ml) (13).

The results suggest that with i.v. formulation or in situations where absorption of oral formulation is more complete, a 5FC dose of 75 mg/kg/day, or even lower, given with AMB, may be associated with maximal additional fungicidal activity.

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