Conversion of 5-Fluorocytosine to 5-Fluorouracil by Human Intestinal Microflora

BARRY E. HARRIS,1 BRADFORD W. MANNING,2 THOMAS W. FEDERLE,2 AND ROBERT B. DIASIO*1

Division of Clinical Pharmacology, Departments of Pharmacology and Medicine,1 and Department of Biology,2 University of Alabama at Birmingham, Birmingham, Alabama 35294

Received 30 August 1985/Accepted 7 October 1985

The mechanism of toxicity from 5-fluorocytosine chemotherapy is unclear. However, recent evidence suggests that the generation of 5-fluorouracil by a host may play an important role in the development of this toxicity. Using an in vitro semicontinuous culture system to mimic the intestinal microflora, we examined the capacity of this complex microbial community to convert 5-fluorocytosine to 5-fluorouracil. The system was dosed initially and after 2 weeks of chronic exposure to 5-fluorocytosine with radiolabeled 5-fluorocytosine. No detectable production of 5-fluorouracil was observed up to 8 h after the acute dose; however, at 24 h and at all time points thereafter, increasing levels of 5-fluorouracil were detected for 4 days. The chronic dose resulted in an increased rate of 5-fluorouracil production without the 8-h lag time. These findings suggest that the enzyme or enzymes responsible for the deamination of 5-fluorocytosine to 5-fluorouracil by the intestinal microflora can be induced by chronic exposure to 5-fluorocytosine and that this conversion may provide a mechanism through which 5-fluorocytosine toxicity is manifested.

5-Fluorocytosine (5-FC; flucytosine) is a fluoropyrimidine that is used primarily in the management of systemic mycoses, such as candidiasis, chromoblastomycosis, cryptococcal meningitis, and touluposporosis (1, 2). Frequent development of resistance to 5-FC during therapy has restricted its use as a single drug in the treatment of fungal infections (4, 14). However, 5-FC can be used effectively in combination with amphotericin B. 5-FC is less toxic than amphotericin B to hosts and can be administered orally (19).

Early pharmacological studies suggested that 5-FC was an ideal drug for the treatment of fungal infections in humans because susceptible fungi contained a cytosine deaminase which converted 5-FC to 5-fluorouracil (5-FU), which was further metabolized to 5-fluorodeoxyuridyllic acid, an inhibitor of thymidylate synthetase and, subsequently, DNA synthesis (18, 19). Host cells lack this enzyme and do not convert large amounts of 5-FC to 5-FU, thus creating a mechanism for the selective toxicity of 5-FC (10, 18). However, some investigators have found that when the serum concentration for 5-FC exceeds 100 μg/ml, patients experience hematologic and gastrointestinal toxicity (2, 11). The symptoms which these patients experience are characteristic of the symptoms that patients develop during 5-FU chemotherapy. In conjunction with this observation, several investigators have reported that patients receiving 5-FC chemotherapy have 5-FU metabolites in their urine (19, 22) and sera (6) at levels comparable to those found during 5-FU chemotherapy (8). Therefore, it seems likely that 5-FU may account for some of the toxicity associated with 5-FC chemotherapy (6).

The mechanism for the conversion of 5-FC to 5-FU in humans is unclear. Previous investigations on the metabolism of 5-FC had varying results. It has been reported that oral administration of 5-FC to rats results in the deamination of 5-FC by the intestinal microflora to 5-FU (12). Although it has been reported that the human intestinal microflora has the capacity to transform a variety of exogenous compounds (9, 20), studies on the metabolism of 5-FC in humans have failed to support the proposal that the human intestinal microflora converts 5-FC to 5-FU. The purpose of this study was to determine whether the human intestinal microflora has the capacity to convert 5-FC to 5-FU.

MATERIALS AND METHODS

Chemicals. 5-FC and 5-FU were purchased from Sigma Chemical Co., St. Louis, Mo. [6-14C]5-FC was obtained from Hoffman-LaRoche, Inc., Nutley, N.J. All other chemicals were analytical grade.

Culture system. An in vitro semicontinuous culture system (15) was used to investigate the intestinal flora-mediated conversion of 5-FC to 5-FU. This system was chosen because of its demonstrated ability for maintaining active anaerobic populations for extended periods of time, as well as its previous successful application to the study of xenobiotic compound metabolism (13, 15). The culture vessel was inoculated with 150 g of freshly voided human feces and maintained in a 37°C water bath under anaerobic conditions. The culture volume was brought to 500 ml with a sterile nutrient suspension which has been described previously (13, 15). The system consisted of a magnetically stirred vessel equipped with ports for gas collection, culture sampling, nutrient addition, and content removal. At 24-h intervals, approximately 40% of the culture was removed; this was followed immediately by the addition of an equal volume of the sterile nutrient suspension. The pulsed delivery of the nutrient suspension was intended to approximate the entry of fluid from the ileum into the large intestine.

Experimental design. Initially, a 5-ml dose of 5-FC (10 mg/ml) and [6-14C]5-FC (100 μCi) was added to the culture via a stopcock-equipped port, resulting in an initial 5-FC concentration of 100 μg/ml and a specific activity of 250 μCi/nmol. Samples were collected via the same port prior to the addition of labeled 5-FC and 2, 4, 8, 24, 48, 72, and 96 h after the introduction of labeled 5-FC into the culture. The culture was subsequently dosed once a day for 15 days with 50 mg of unlabeled 5-FC. On day 16 the culture was dosed with 50 mg of 5-FC and 50 μCi of [6-14C]5-FC. Again, samples were taken prior to the addition of labeled 5-FC and
at 2, 4, 8, 24, 48, 72, and 96 h following the addition of labeled 5-FC.

All samples were centrifuged at 2,000 × g in an IEC model B-20A centrifuge (Damon/IEC Div., Needham Heights, Mass.) for 20 min at 4°C. The resulting supernatant was passed through a 0.45-μm filter and analyzed for 5-FC and its metabolites by high-pressure liquid chromatography (HPLC).

Identification of metabolites. Reverse-phase HPLC was performed with a model 1084A liquid chromatograph (Hewlett-Packard Co., Avondale, Pa.) equipped with an automatic injector system and a 254-mm fixed-wavelength detector. Metabolites were separated on 5 μM C18 reverse-phase column (25 by 0.45 cm; IBM Instruments, Inc., Poughkeepsie, N.Y.) by isocratic elution with a buffer containing 5.0 mM tetrabutylammonium hydrogen sulfate and 1.5 mM potassium dihydrogen phosphate adjusted to pH 8.0. The flow rate was 1.0 ml/min. The eluent was monitored at 254 nm and recorded at an attenuation of 0.384 absorbance unit (full scale). The integrator slope sensitivity was 0.2. Fractions were collected at 1.0-min intervals, 5.5 ml of a scintillation solution (Triton X-100-type 3A20 counting cocktail, 1:2; Research Products International, Mount Prospect, Ill.) was added to each fraction, and the preparations were mixed thoroughly. The total amount of [6-14C]5-FC or its metabolites in each fraction was determined by using a model LS5801 liquid scintillation counter (Beckman Instruments, Inc., Irvine, Calif.).

By using the HPLC method described above, 5-FC was found to have a retention time of 6.5 min (k’ = 0.26). 5-FU was completely resolved from 5-FC and had a retention time of 14.3 min (k’ = 1.67) (Fig. 1).

Analysis of fermentation products. The presence of an active anaerobic community within the culture for the duration of the experiment was confirmed by the detection of volatile fatty acids (VFAs) and methane. Formate, acetate, propionate, butyrate, and lactate were analyzed before and after the addition of 5-FC by a modification of the method described by Bethge and Lindstrom (3, 13). Gas was collected once before and several times after the addition of 5-FC and analyzed for the presence of methane.

RESULTS

The in vitro semicontinuous culture system maintained an active anaerobic community throughout the experimental period, as verified by the formation of fermentation products (Table 1). Our in vitro fermentation approximated the fermentation believed to occur in vivo, with the relative amount of each VFA analyzed similar to the amount found in feces (5, 23). Anaerobic roll tube and standard plate counts were also performed on three separate occasions during the experimental period; these counts averaged 1.01 × 10⁹ and 3.4 × 10⁸ bacteria per ml, respectively. Therefore, the ratio of total anaerobes to facultative anaerobes was 297:1.

The absolute and relative amounts of fermentation products detected after the addition of 5-FC were not significantly different from the amounts present before the addition of 5-FC. This suggests that 5-FC had little or no effect on the overall microbial activity. These data were also in general agreement with previous results obtained with this system. Over an 8-day period, Miller and Wolin (15) reported that acetate, propionate, butyrate, and formate accounted for 76,

<table>
<thead>
<tr>
<th>Compound</th>
<th>Before 5-FC treatment</th>
<th>After 5-FC treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amt produced (mmol/liter)</td>
<td>% of total VFAs</td>
</tr>
<tr>
<td>Formate</td>
<td>0.87 ± 0.56</td>
<td>1.19</td>
</tr>
<tr>
<td>Acetate</td>
<td>54.38 ± 4.15</td>
<td>75.00</td>
</tr>
<tr>
<td>Propionate</td>
<td>11.10 ± 0.64</td>
<td>15.31</td>
</tr>
<tr>
<td>Butyrate</td>
<td>6.17 ± 0.84</td>
<td>8.50</td>
</tr>
<tr>
<td>Lactate</td>
<td>ND</td>
<td>0.01 ± 0.02</td>
</tr>
</tbody>
</table>

* Calculated from eight daily determinations.
* Calculated from 15 daily determinations.
* Mean ± standard deviation.
* ND, Not detected.
* Lactate was detected in only 4 of the 15 samples; the mean was calculated by dividing the total amount of lactate by 15.
13, 10, and 1% of the total VFAs, respectively, whereas we found that these VFAs accounted for 75, 15, 9, and 1% of the total VFAs recovered, respectively (Table 1). Lactate was assayed for by Miller and Wolin but was not detected. We did not detect any lactate in our initial culture but found low levels of lactate in 4 of the 15 samples analyzed after the addition of 5-FC. Our detection of small quantities of lactate may be attributable to the greater sensitivity of the method used in this study compared with the colorimetric assay used by Miller and Wolin.

The radiometric profile obtained through separation by HPLC for each sample was used to determine the amounts of labeled 5-FC and its metabolites in the sample. 5-FU was the only metabolite which accounted for more than 1% of the recovered radioactivity. A typical radiometric profile is shown in Fig. 1B.

Figure 2 shows the relative concentrations of 5-FC and 5-FU expressed as micrograms of drug per milliliter of culture at each time point. The concentrations of 5-FC and 5-FU were calculated from the total amount of radioactivity recovered for each compound and the specific activity at which the system was dosed with labeled 5-FC. Daily samples were corrected for removal and dilution for both the acute and chronic doses. The values obtained at 0, 2, 4, 8, and 24 h were not corrected; those obtained at 48, 72, and 96 h were corrected for removal and dilution of the system by a factor which was calculated by dividing the total number of disintegrations per minute recovered at each time point by the total number of disintegrations per minute recovered at the 24-h time point. In both sets of data, the calculated value of this correction factor approximated the expected theoretical value.

Figure 2 shows that very little 5-FU was formed in the first 8 h after the acute dose; however, after the initial 8 h, the 5-FU concentration increased steadily for 4 days without reaching a peak. After 2 weeks of daily 50-mg doses of unlabeled 5-FC, there was no lag in the formation of 5-FU, and the rate of formation was nearly 2.5 times the rate observed during acute exposure. With the acute dose, the 5-FU concentration after 4 days was 9.42 \( \mu g/ml \) of culture, which represented approximately 7.5% of the total radioactivity recovered; with the chronic dose, the 5-FU concentration was 31.86 \( \mu g/ml \) of culture, representing approximately 20.0% of the total radioactivity.

**DISCUSSION**

Previous studies on the metabolism of 5-FC in humans have yielded varying results and no definite conclusions. Some investigators contend that more than 95% of orally administered 5-FC is excreted into the urine and feces unchanged, with no detectable amount of 5-FU in the patients studied (12, 17). Other workers have reported detectable levels of 5-FU in the urine (19, 21) and sera (6) of patients receiving 5-FC chemotherapy.

Whatever the mechanism of pathogenesis of 5-FC, it is obvious that clinical toxicity of 5-FC exists. Bone marrow suppression leading to anemia, leukopenia, and thrombocytopenia occurs in approximately one-third of all patients receiving 5-FC chemotherapy (2, 21). Liver abnormalities and gastrointestinal ailments occur in less than 10% of patients during therapy (2, 21). Also, 5-FU toxicity is clearly associated with serum levels greater than 100 \( \mu g/ml \) (2, 11).

It is conceivable that 5-FU could contribute to the clinical toxicity manifested during 5-FC chemotherapy through release of 5-FU from killed fungi and subsequent resorption into human cells. Previous investigations have not addressed this question, and as it is without precedent, it appears to be an unlikely mechanism for appearance of 5-FU in patient serum and urine during therapy. It has been suggested that the human microflora might play a role in the conversion of 5-FC to 5-FU (12). Certain microorganisms found in normal intestinal microflora, such as *Lactobacillus* and *Bacteroides*, may not possess cytosine deaminase as a constitutive enzyme. However, *Escherichia coli* has been shown to have extremely high cytosine deaminase activity (16). Enterococci and enterobacteria (such as *E. coli*) are readily culti-
vated in the microflora, and bacteriological studies have shown that these organisms are present in the intestinal microflora (7). In this study we evaluated the capacity of the human microflora to convert 5-FC to 5-FU, thus providing a mechanism for 5-FC absorption into the sera of patients undergoing 5-FC chemotherapy.

Over the past 25 years, the human microflora has received much attention with respect to the metabolism of xenobiotic compounds. It has become apparent that the intestinal microflora plays an important role in the pharmacological and toxicological effects of many drugs, carcinogens, and other xenogenous compounds (9). In 1981, Miller and Wolin (15) developed an in vitro semicontinuous culture system which maintained a diverse and complex community with activities resembling those observed in vivo. In previous studies of 5-FC metabolism in humans workers examined the levels of 5-FU and its metabolites in sera, urine, and feces. In these studies the absence of detectable levels of 5-FU or its metabolites led the investigators to assume that there was no metabolism of 5-FC. With the system developed by Miller and Wolin, we could directly determine the capacity of the human intestinal microflora to deaminate 5-FC to 5-FU.

In our study we measured the metabolism of 5-FC in our culture system after acute and chronic exposures to 5-FC. Acute exposure resulted in a lag time of 8 h and a small amount of metabolism which continued as long as there was 5-FC present in the system. A single dose of labeled 5-FC after chronic exposure yielded somewhat different results in that there was no lag time and the rate of metabolism was greatly increased (Fig. 2). This difference between acute exposure and chronic exposure to 5-FC may have been due to induction of the enzyme or enzymes responsible for the conversion of 5-FC to 5-FU or to a shift in the community composition which may have predisposed the flora to convert 5-FC to 5-FU. In a recent clinical study in which 5-FC toxicity was evaluated, it was found that toxicity did not correlate with isolated serum levels of 5-FC above 100 μg/ml, but was more likely to occur when high levels were present for 2 or more weeks of therapy (A. M. Stamm et al., manuscript in preparation).

In addition to our findings, it would be of interest to determine the effect of concurrent administration of amphotericin B with daily doses of 5-FC on the metabolism of 5-FC by the microflora. Clinically, 5-FC is given concurrently with amphotericin B for most fungal infections. Many of the patients receiving 5-FC chemotherapy have undergone previous antibiotic therapy and therefore may be subject to an alteration of their intestinal microflora. This alteration may affect the intestinal flora-mediated conversion of 5-FC to 5-FU and, therefore, could explain the variability observed in clinical applications of 5-FC. Also, conjunctive antibiotic therapy needs to be investigated as a possible modulator of 5-FC toxicity. With the proper regimen, conjunctive antibiotic therapy may be used to decrease the activity of selected microflora and thereby decrease the conversion of 5-FC to 5-FU and 5-FU-associated toxicity.

In summary, our results illustrate the applicability of the semicontinuous culture system for investigating the metabolism of xenobiotic compounds by the human intestinal microflora. It is evident that the human intestinal microflora plays an important role in the pharmacological and toxicological effects of many therapeutic compounds. Its role in the 5-FU-associated toxicity secondary to 5-FC treatment seems apparent in that it provides a mechanism for the metabolism of 5-FC to 5-FU. In addition, an interesting question arose from the effect of chronic exposure of a xenobiotic com-

CONVERSION OF 5-FC TO 5-FU IN HUMANS

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grant CA-40530 from the National Cancer Institute. The typing assistance of Patricia Pierce is greatly appreciated.

LITERATURE CITED