Bioavailability Study of Oral Liquid and Tablet Forms of α-Difluoromethylornithine

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ABSTRACT

The purpose of this study was to assess the bioavailability of two oral preparations of difluoromethylornithine (DFMO). The current preparation of DFMO is a liquid with a concentration of 0.2 gram/ml that must be drawn up into a syringe and dispensed into a small medicine glass. This form of DFMO causes wastage of the medication. The liquid form also makes compliance and blinding difficult. Recently, a new coated tablet preparation has become available from Ilex Oncology Services (San Antonio, TX). The coated tablets are 0.25 gram and are scored. This form should increase compliance by making it much easier for the subject to take the medication. This report compares the bioavailability of both preparations with the aim of demonstrating equivalence of the preparations. Ten normal subjects entered the cross-over study in which the order in which they would receive the liquid or tablet preparation of DFMO was randomized. The study was designed with the objective of establishing the bioequivalence of a tablet preparation of DFMO at daily dose 0.5 gram/m² and a liquid preparation of DFMO at the same daily dose. The study was designed with the objective of establishing the bioequivalence of a tablet preparation of DFMO at daily dose 0.5 gram/m² and a liquid preparation of DFMO at the same daily dose. The mean area under the time-by-concentration curves (µM × hours) for the liquid and tablet preparations was 368.2 and 370.4, respectively. The peak concentrations for the liquid and tablet preparations were 47.3 and 48.2 µM, respectively. No statistically significant differences were seen in these parameters, in time to peak concentration, or in serum half-life.

The criterion for bioequivalence of the two preparations was satisfied.

INTRODUCTION

DFMO is an enzyme-activated, irreversible inhibitor of ODC that acts by covalently binding to the enzyme (1). DFMO in animals prevents experimentally induced skin, breast, colon, intestinal, and bladder cancers (1–3).

Studies of DFMO have shown that despite a serum half-life of 2–4 h, DFMO given once a day decreases ODC measured in skin biopsies that is maintained for more than 10 months (4). In this study, the lowest total daily dose administered was 0.5 gram/m²/day, which was proven to be safe and effective. These data are in agreement with experimental data in mice that showed a marked inhibition of ODC skin activity by small DFMO doses that persists for 5 days after DFMO is discontinued. After 72 h, the enzyme activity was still 50% of baseline (5). Loprinzi and Verma (5) calculated that the dose used in their experiments of 1 mg/mouse is equivalent to 0.1–0.15 gram/m² in humans.

Although a tablet form has been available, no human pharmacokinetic studies have been done with this preparation. In an ongoing large Phase III trial at the University of Wisconsin, 334 subjects with skin cancer have been randomized to DFMO or a placebo using the liquid preparation. The current preparation of DFMO is a liquid with a concentration of 0.2 gram/ml that must be drawn up into a syringe and dispensed into a small medicine glass. The liquid is then mixed with fruit juice to help mask the taste before it is taken by the subject. This form of DFMO causes wastage of the medication. The liquid form also makes compliance and blinding difficult. Recently, a new coated tablet preparation has become available from Ilex Oncology Services (San Antonio, TX). The coated tablets are 0.25 gram and are scored. This form should increase compliance by making it easier for the subject to take the medication. Taste would also be more acceptable. This report compares the bioavailability of both preparations with the aim of demonstrating equivalence of the preparations.

PATIENTS AND METHODS

Design. Ten normal subjects entered the cross-over study, in which the order in which they received the liquid or coated tablet preparation of DFMO was randomized. Doses were rounded to the nearest 250 mg based on the tablet dose. On day 1, subjects received the preparation to which they were randomized and had plasma concentration measurements taken

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3 The abbreviations used are: DFMO, α-difluoromethylornithine; ODC, ornithine decarboxylase; AUC, area under the time-by-concentration curves; FDA, Food and Drug Administration.

4 R. Jacoby, personal communication.
molecular formula is C₆H₁₂N₂O₂F₂HCl,H₂O. Its molecular concentration
through the week following the second dose of medication. The subjects then received the opposite preparation of DFMO than those recorded, and toxicity assessments were performed. The clinical status (performance status, weight, blood pressure, headache, nausea, and diarrhea). The research staff evaluated the frequency of common health-related complaints (i.e., fatigue, headache, nausea, and diarrhea). The research staff evaluated the subjects’ clinical status (performance status, height, weight, blood pressure, pulse, respiration, and temperature) and obtained a list of concomitant medications. Subjects were then randomized to receive one of the two formulations of DFMO, liquid or tablet.

On the day 8 visit, the research staff evaluated subjects’ clinical status (performance status, weight, blood pressure, pulse, respiration, and temperature). Concomitant medications were recorded, and toxicity assessments were performed. The subjects then received the opposite preparation of DFMO than that taken on day 1.

On day 15, subjects were called by study personnel. Subjects were asked about any side effects that may have occurred during the week following the second dose of medication. Concomitant medications were also updated.

**Sampling Procedures.** Pharmacokinetic blood sampling was performed on days 1 and 8 via repeated venipuncture or an indwelling heparin lock catheter. At each sampling point, 3 ml of blood were drawn and discarded prior to taking a 10-ml sample. Blood samples were taken at the following times: before drug administration (0); 30, 60, and 90 min after drug administration; and 2, 3, 4, 6, 9, 22, and 24 h after drug administration.

**Pharmacological Information.** DFMO, an antiparasitic drug, is used in the treatment of cancer and Trypanosoma brucei gambiense sleeping sickness. The chemical name is 2-(difluoromethyl)-3,1-ornithine monohydrate. The molecular formula is C₆H₁₂N₂O₂F₂HCl,H₂O. Its molecular weight is 236.65.

The mode of action of DFMO has been attributed to its inhibitory action on the enzyme ODC. It is a specific, enzyme-activated, irreversible inhibitor of ODC that regulates the biosynthesis of polyamines in all mammalian as well as many other eukaryotic cells (6). By its inhibition of ODC and the subsequent synthesis of polyamines, DFMO inhibits the growth of many cell types, especially those that are rapidly dividing and have an obligatory need for polyamine synthesis, i.e., tumor cells.

The liquid preparation of DFMO has been the subject of pharmacokinetic and pharmaco logical studies in man (4). Single-dose pharmacokinetics are linear at all dose levels. Steady-state trough plasma concentrations were proportional to dose. About 50% of the p.o. administered DFMO is absorbed, and 86% is eliminated unchanged in the urine. The most common adverse reactions include gastrointestinal toxicity (mild diarrhea, abdominal pain, anorexia, and nausea) and ototoxicity (hearing loss and tinnitus).

Liquid DFMO is supplied as a bulk aqueous solution containing 200 mg of active drug per milliliter of solution. The solution should be stored at a controlled room temperature. DFMO is supplied in tablets of 250 mg/tablet. The tablets are stable at room temperature.

**DFMO Pharmacokinetics.** DFMO in plasma was assayed by high-performance liquid chromatography using a procedure similar to that described by Smithers (7). Plasma samples (0.1 ml) were extracted with 4 volumes of methanol after addition of the internal 4-amino-3-hydroxybutyric acid. The extracts were derivatized with o-phthalaldehyde, and chromatographic separation of the o-phthalaldehyde-derivated samples was achieved using a Waters Nova-Pak cartridge, gradient elution with a methanol-phosphate buffer (pH 7.5) solvent system, and fluorescent detection (335 nm excitation and 450 nm emission). Quantitation was done by comparing the peak height of DFMO to that of the internal standard.

**Statistical Analysis.** The primary end point was the AUC. These areas were computed using a trapezoidal approximation with concentration measurements at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 9, 22, and 24 h. Other end points included peak plasma concentration, time to peak concentration, and half-life. The means of each parameter were summarized for each dose and preparation, along with confidence intervals for the differences between these parameters for the two preparations. For assessment of bioequivalence, a confidence interval for the difference between mean [log AUC (tablet)] and mean [log AUC (liquid)] was computed. An interval that fell entirely within some tolerance limits was taken as sufficient evidence that for the dose being studied, the tablet preparation and a 0.5 gram/m² dose of the liquid preparation are bioequivalent with respect to their bioavailability. For this study, we required that the AUC for the tablet preparation differ by no more than 33% of the AUC for the liquid preparation, as shown by the equation below.

\[ 0.67 \leq \frac{\text{AUC (tablet)}}{\text{AUC (liquid)}} < 1.33 \]
the difference in the mean log AUCs. The following bioequivalence hypotheses were tested.

Alternative: \( \log(0.67) < \text{mean} [\log(\text{AUC (tablet)})/\text{AUC (liquid))}] < \log(1.33) \) versus the null hypothesis that the difference between the mean log AUCs does not fall within these limits. Bioequivalence would be decided if a 95% confidence interval for this parameter fell entirely within the interval \( [\log(0.67), \log(1.33)] \).

**RESULTS**

**Toxicity.** We recorded all of the side effects experienced by the subjects according to the National Cancer Institute Revised Common Toxicity Criteria (version 2). Based on a review by the study chair and the clinical nurse specialists, we assigned the relative degree of likelihood that the toxicities were drug related (unrelated, unlikely, possibly, probably, and definite). No grade 2 or higher toxicities were observed, and no single grade 1 toxicity was reported by more than one subject. The reported grade 1 toxicities were cold symptoms and urinary frequency (unlikely); excessive thirst, canker sore, and rash (unlikely); and heartburn (possibly).

**Pharmacokinetic Results.** The mean values of AUC, peak concentration \( (C_{\text{max}}) \), time to peak concentration \( (T_{\text{max}}) \), and half-life for the 10 subjects are shown in Table 1. All parameter estimates are very similar for the two preparations, and there were no statistically significant differences between the means of the two formulations, as determined by using a paired \( t \) test at a significance level of 0.05. As described in the statistical analysis section, bioequivalence was determined by examining a 95% confidence interval for the following parameter: mean \( [\log(\text{AUC (tablet)})] - \text{mean} [\log(\text{AUC (liquid)})] \). The confidence interval for this difference is \(-0.185 \) to \( 0.162 \), which is contained in the interval \( [\log(0.67), \log(1.33)] \). Accordingly, we concluded that the two formulations are bioequivalent. Also, the 95% confidence interval for the mean of the ratio AUC (tablet)/AUC (liquid) on the original scale \( (\mu \text{M} \times \text{h}) \) is \( 0.85 \pm 1.17 \), which falls within the interval of 33% tolerance \( (0.67 \pm 1.33) \). Furthermore, Fig. 1 reveals that mean plasma concentrations of DFMO were nearly identical at each time point. Because of the sharp drop in concentration for both preparations that occurs between 6 and 9 h, it is possible that the trapezoidal rule overestimates the true AUCs for both preparations when the next measurement is taken at 22 h. However, Fig. 1 reveals that the bias in estimation is likely to be the same for each preparation.

**DISCUSSION**

DFMO as an antitumor agent has not been highly effective. Doses of DFMO were given on an intermittent schedule to patients with metastatic breast cancer with no significant objective antitumor response (8). In this study, 4800 mg were administered four times a day for 14 days with a 2-week drug holiday without ototoxicity. A study by Ajani et al. (9) of colorectal cancer showed that i.v. continuous infusion DFMO therapy resulted in only mild gastrointestinal toxicity. DFMO proved to be ineffective as a single agent in this trial. Levin et al. (10) treated 80 patients (36 glioblastoma multiforme patients and 44 anaplastic glioma patients). Antitumor activity (partial response, minor response, and stable disease) was seen in 45% of the patients with anaplastic gliomas for a median of 49 weeks but was seen in only 17% of patients with glioblastoma multiforme. Interest in this compound has been revived because of its activity in many preclinical models of chemoprevention where lower doses and long-term therapy of precancer lesions are the objectives.

ODC is essential for polyamine synthesis and growth in mammalian cells. Many highly specific inhibitors of ODC are based on DFMO, which is an enzyme-activated irreversible inhibitor. DFMO is accepted as a substrate by ODC and is decarboxylated, leading to the formation of a highly reactive species that forms a covalent adduct with either cysteine 360 (90%) or lysine 69 (10%; Ref.11). Both modifications inactivate the enzyme. Along with studies showing that many tumor promoters increase ODC activity and that a number of preneoplastic conditions and tumor samples show high levels of ODC, these results suggest that ODC may act as an oncogene in an appropriate background. This provides a rationale for the possible use of ODC inhibitors as chemopreventive agents. DFMO inhibits tumor formation in experimental models of bladder, colon, breast, liver, stomach, and skin cancers (12–15). DFMO has also been shown to inhibit polyp formation in animals with a min gene defect.4

### Table 1 DFMO pharmacokinetic results (mean ± SE)

<table>
<thead>
<tr>
<th>DFMO preparation</th>
<th>AUC (( \mu \text{M} \times \text{h} ))</th>
<th>( C_{\text{max}} ) (( \mu \text{M} ))</th>
<th>( T_{\text{max}} ) (h)</th>
<th>Half-Life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liquid (0.5 gram/m²)</td>
<td>368.2 ± 16.6</td>
<td>47.3 ± 1.6</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Tablet (0.5 gram/m²)</td>
<td>370.4 ± 26.4</td>
<td>48.2 ± 2.2</td>
<td>3.9 ± 0.4</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>( P ) (paired ( t ) test)</td>
<td>0.894</td>
<td>0.712</td>
<td>0.299</td>
<td>0.650</td>
</tr>
</tbody>
</table>

![Fig. 1 Mean time (hour) versus concentration (micromolar) curves are plotted for the coated tablet preparation (solid line) and the liquid preparation (dotted line).](image-url)
Elevated polyamine levels are characteristic of many types of neoplastic cells and tissues. In transgenic mice overexpressing ODC in skin, changes in tissue polyamine levels, particularly putrescine, control the development and maintenance of the neoplastic phenotype (16). DFMO, a specific inhibitor of the transgene, reversibly blocked the appearance of squamous papillomas after carcinogen treatment. Furthermore, treatment of papilloma-bearing mice with DFMO caused rapid tumor regression, also in a reversible manner. Tumor cell proliferation was rapidly decreased after drug treatment. Conversely, proliferation of normal epidermal keratinocytes was unaffected by DFMO treatment. Peralt Soler et al. (16) concluded that polyamine levels are required for both the development and maintenance of the neoplastic phenotype in skin.

ODC is a key enzyme in mammals for the biosynthesis of polyamines, putrescine, spermidine, and spermine. It is barely detectable in normal tissues but is rapidly inducible in normal tissues and has a half-life of <20 min (6, 17).

The $t_{1/2}$ of DFMO is only 4 h. The question of whether a single daily oral dose of DFMO would have a persistent biological effect is relevant. In cell culture, ODC inhibition by DFMO lasts 48 h (18, 19). Creaven et al. (20) have shown that even at doses of 0.2 gram/m2/day, serum concentrations of DFMO are achieved that are able to inhibit ODC in cell culture systems. Verma (21) has shown that the ODC mRNA persists for a much longer time. Ishiwata et al. (22) have shown that DFMO is sequestered in the cells and can inhibit ODC for longer periods of time. Schedules of some of the polyamine ODC inhibitors in which drug is given for 4 days and then rested for 3 days have shown significant biochemical and chemopreventive effects with lessened overall toxicity.

Our own human studies of DFMO have shown that despite a serum half-life of 2–4 h, DFMO given once a day causes a decrease in ODC measured in skin biopsies that is maintained for more than 10 months (4). In this study, the lowest total daily dose administered was 0.5 gram/m2/day. These data are in agreement with experimental data in mice that showed a marked inhibition of ODC skin activity by small DFMO doses that persisted for 5 days after DFMO was discontinued. After 72 h, the activity was still 50% of baseline (5). Loprinzi and Verma (5) calculated that the dose used in their experiments of 1 mg/mouse would be equivalent to 0.1–0.15 gram/m2 in humans.

Until now, the only preparation that has been used in human studies has been a liquid preparation that contains 0.2 gram/ml solution in 475-ml plastic bottles. Subjects have been instructed to draw the liquid up into a plastic syringe and dispense the liquid into a small medicine glass. Subjects were then instructed to dilute the material in a fruit juice to mask the taste. This led to problems with wastage of material and allowed for a potential error in dosage. Likewise, the preparation of a placebo control was difficult. A tablet has recently become available that was approved by the FDA for use in a bladder cancer prevention trial cosponsored by Ilex Oncology Services and the National Cancer Institute. However, animal data were obtained, but no human pharmacology was done. The FDA accepted the dosage form based on animal studies of toxicity. The current bioavailability study was done because we had started a large Phase III chemoprevention trial in skin cancer with the liquid preparation. We wanted to convert to the tablet for the convenience of our subjects and to encourage compliance over the 4-year treatment duration of our study. The current data were helpful in convincing the sponsors, the Data Monitoring Safety Committee, and the FDA that using the pill would not compromise the research protocol. Our study indicates that liquid DFMO and tablet DFMO administered at 0.5 gram/m2 have nearly identical pharmacokinetic parameters, and population bioequivalence was easily established under the criterion we adopted.

Our pharmacokinetic studies of DFMO have shown that the tablet form of DFMO is equivalent to the liquid preparation. The mean values of AUC, peak concentration ($C_{\text{max}}$), time to peak concentration ($T_{\text{max}}$), and half-life for the estimates are very similar for the two preparations. There were no statistically significant differences between the means of the two formulations. These data were sufficient to convince the Data Monitoring Safety Committee, the sponsors, and the FDA that changing to the tablet formulation would not compromise our study.

A search of the CRISP (NIH) database of grants reveals at least five human Phase II or III trials using DFMO as a chemoprevention agent. These trials include non-melanoma basal cell cancers, early bladder cancer, breast cancer, anal carcinoma in HIV-positive men, and prostate cancer. Experimental data also suggest that DFMO might be useful in treatment of a variety of other tumors. The demonstration of an adequate oral tablet preparation should make these trials more cost effective and ensure better compliance.

REFERENCES


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