Pharmacokinetics of the Chemopreventive Agent Oltipraz and of Its Metabolite M3 in Human Subjects after a Single Oral Dose

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ABSTRACT

The dithiolethione oltipraz (OPZ) has activity as a chemopreventive agent in animal models and is in early clinical trials. OPZ undergoes metabolism by molecular rearrangement to yield a pyrrolopyrazine derivative, M3, which we have previously shown to be active in the induction of detoxication genes. M3 is metabolized further: at least 10 possible conjugates have been described in three species. We developed a new high-performance liquid chromatography method to simultaneously measure plasma concentrations of OPZ and of M3. This method was applied to serial plasma samples in a Phase I clinical trial, in which OPZ was administered at single doses varying from 125 to 1000 mg/m². OPZ and M3 concentration-time profiles were highly variable among individuals, and the occurrence of secondary concentration peaks suggested substantial enterohepatic cycling. Absorption was rapid, and the mean time to peak was 2.2 h. Maximum plasma concentration values were proportional to the dose. Harmonic mean half-lives at these doses ranged from 9.3–22.7 h. There were indications of dose-dependent pharmacokinetic properties because apparent clearance and volume of distribution at steady state increased with dose, although these changes were not statistically significant as a result of high interpatient variability. Accordingly, there were less than proportional increases in the OPZ and M3 area under the curve and maximum plasma values. Interpretation of OPZ and M3 disposition is confounded by the unknown bioavailability factor; however, the most likely inferences are that bioavailability of OPZ decreases with increasing dose and that metabolism to M3 is saturable.

INTRODUCTION

A high intake of cruciferous vegetables (including cabbage, broccoli, and cauliflower) is associated with protection from the development of colorectal cancer (1–3). These plants all contain substantial concentrations of dithiolethiones, indoles, and isothiocyanates, each of which has been proposed to account for chemoprotection (4). A synthetic dithiolethione, OPZ, was originally developed for the treatment of schistosomiasis (5). Its ready availability led to testing in models of carcinogenesis. Administration of OPZ in the diet was found to protect rodents from the formation of carcinogen-induced tumors at various sites (6–8). Accordingly, it has been developed in the clinic as a candidate chemoprevention drug.

OPZ is believed to exert its protective effects through the induction of phase II detoxicating enzymes in the liver and other organs (9, 10). Evidence suggests that transcriptional induction underlies this effect, but the signaling pathways involved remain obscure (11–13). In addition, alternative mechanisms of protection involving stimulation of DNA repair have been described previously (14).

Induction of detoxication genes by OPZ appears to depend on an intact dithiolethione ring structure (11, 12). We have previously shown that the metabolite of OPZ M3, a pyrrolopyrazine molecular rearrangement, is inactive in assays of transcription induction in vitro, whereas the keto derivative M2 retains all the activity of the parent compound (12). Hence the metabolism of OPZ to M3 represents an inactivating step. In a clinical trial of single dose oltipraz with biological end points, we wished to measure the plasma pharmacokinetics of OPZ and of this major metabolite M3 as a means to quantitate this inactivation. A new HPLC assay was developed for this purpose and is reported together with the pharmacokinetics of OPZ and M3.

PATIENTS AND METHODS

Chemicals. OPZ and the internal standard, anethole tri-thione, were obtained from Dr. M. L. Clapper (Division of Population Science, Fox Chase Cancer Center). The metabolites 6,8-dimethylthio-7-methylpyrrolo[1,2-alpyrazine (M3) and 4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-one (M2) were synthesized in the Organic Synthesis Facility, Fox Chase Cancer Center. High purity water was produced with a Nanopure ultra-pure water system (Barnstead, Dubuque, IA). All other chemical and solvents were of analytical grade or higher and purchased from commercial sources.

Study Population. Eligible subjects for this study were at increased risk for colorectal carcinoma and included men and women with the following characteristics: age 40–70 years, at least one first-degree relative with colorectal carcinoma, and a minimum age at diagnosis of 50 years. Subjects were required to have normal liver function tests, hematology, and coagulability. The study was approved by the Institutional Review Board at the University of Pennsylvania and satisfied all regulations of the United States Food and Drug Administration and the Committee on Human Research of the University of Pennsylvania.

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women over the age of 18 years with a family history of colorectal cancer, a personal history of colon polyps, or a personal history of colorectal cancer (≥3 months from definitive treatment). The characteristics of the population treated are described in more detail elsewhere (15). All patients had a medical history, physical examination, complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, biochemical profile, urine analysis, and electrocardiogram. All eligible patients were physiologically normal, as evidenced by an Eastern Cooperative Oncology Group performance status of 0 and adequate bone marrow, renal, and liver function. Subjects were asked to refrain from the use of aspirin, nonsteroidal anti-inflammatory drugs, and corticosteroids during the study time period. Patients gave written informed consent in accordance with federal, state, and institutional guidelines.

**Treatment Plan.** Twenty-four eligible high-risk individuals were enrolled and treated with doses of OPZ ranging from 125–1000 mg/m². After an overnight fast, the assigned dose of OPZ (rounded to the nearest 20 mg) was administered orally with 8 ounces of water under supervision. OPZ was synthesized by Rhone-Poulenc Rorer (Vitry-sur-Seine, France) and supplied for this trial by the Division of Cancer Prevention and Control (Bethesda, MD) in capsules that ranged in size from 20–250 mg. Subjects were permitted to begin eating and drinking 5 h after dosing.

Serial blood samples were collected into heparinized tubes at 0 (predose), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12, 18, and 24 h after dosing. Plasma was harvested by centrifugation (5 min at 3000 rpm) and stored at −80°C until analyzed by HPLC.

**Analysis of OPZ and M3 in Plasma and Urine.** Patient plasma samples of 1 ml containing 50 µl of internal standard solution (either 3 or 30 µg/ml) in 13 × 100-mm borosilicate glass tubes with Teflon-lined caps were extracted with 3 ml of cyclohexane by gentle mixing for 15 min. The tubes were then centrifuged for 10 min at 3500 × g and placed in a −80°C freezer for 10 min to facilitate decanting of the upper organic layer into clean glass tubes, in which the organic phase was dried under nitrogen gas at 45°C for 20 min. The residue was reconstituted in 300 µl of 50% methanol and subsequently used for the HPLC analysis.

The HPLC system consisted of a Hewlett-Packard (Palo Alto, CA) HP1090A liquid chromatograph equipped with an autoinjector and a diode-array UV detector. The detector wavelength was initially set at 290 nm and then switched to 302 nm at 4 min and to 248 nm at 8 min for each run to optimize the response for each analyte. Chromatographic separation was achieved on a C8 reversed-phase column (Adsorbosphere; 5 m; 150 × 4.6 mm inner diameter; Alltech Associates, Deerfield, IL) preceded by a guard column of the same stationary phase (10 × 4.6 mm). An isotropic separation of analytes was achieved using a mobile phase that consisted of 58% 0.005 M dimethylformamide, 0.0005 M sodium acetate (pH 5.5), and 42% acetonitrile at a flow rate of 1.5 ml/min. The retention times were 5 min for OPZ, 10 min for M3, and 12 min for the internal standard.

Calibration curves were linear over a OPZ concentration range of 10–1000 ng/ml and over a range of 10–250 ng/ml for M3. The lower limit of detection was 2 ng/ml for both OPZ and M3. Accuracy and precision of the assay procedure were indicated by quality control samples included at three different OPZ and M3 concentrations for each calibration curve. The quality control samples were within 20% bias and coefficient of variation.

**Pharmacokinetic Analysis.** Of the 24 patients enrolled in the study, 18 patients had sufficient OPZ plasma concentration measurements to perform the following pharmacokinetic analyses. Fourteen of these 18 patients provided complete M3 concentration-time data for the associated M3 pharmacokinetic analyses. OPZ plasma concentration-time data were analyzed by noncompartmental analysis (16) to yield estimates of the AUC, area under the first moment curve (AUMC), apparent total clearance (CL/F), apparent volume of distribution at steady state (Vss/F), and the terminal elimination half-life (t1/2). AUC and AUMC were calculated by Lagrange polynomial interpolation to the last measured time and with extrapolation to time infinity based on the terminal disposition rate constant obtained by log-linear regression of the terminal linear segment. For M3, AUC and t1/2 were determined. The observed time (tmax) of the maximum plasma concentrations (Cmax) was recorded as the first peak for both OPZ and M3. In all but a minority of subjects, second OPZ and M3 concentration peaks were observed. The ratio of AUCM3/AUCOPZ was calculated for each patient. ANOVAs were used to determine differences in the pharmacokinetic parameters as a function of dose. All analyses were completed with the JMP statistical software package (17).

**RESULTS**

A new HPLC method was developed that allowed the simultaneous measurement of OPZ, M3, and M2. Similar to previous HPLC methods, a liquid-liquid extraction from plasma was used, but a more hydrophilic analytical column facilitated the separation and quantitation of the solutes in a relatively short time frame. Although OPZ and M3 could be measured in all patients, M2 could not, and where a signal could be identified, it was near the limit of assay sensitivity.

In general, OPZ plasma concentration-time profiles displayed a large degree of intersubject variability. In addition, many patients showed secondary concentration peaks (Fig. 1). Table 1 provides OPZ pharmacokinetic parameters for each dose level. Neither CL/F nor Vss/F showed a significant dependence on dose, consistent with linear or dose-independent pharmacokinetics (Fig. 2). However, values for CL/F varied more than 2-fold over doses from 100–1000 mg/m² (Fig. 3). Vss/F values demonstrated an increasing trend as dose increased. There were less than proportional increases in AUC and Cmax values with increasing dose, suggestive of a saturable phenomenon. Drug absorption was rapid based on an observed tmax of about 2 h, corresponding to the first concentration peak. There is over a 2-fold range in tmax values, with values decreasing as dose increased. Again, because of the large intersubject variability, this trend was not statistically significant.

Concentrations of M2 were close to the limit of detection and could not be characterized kinetically. Pharmacokinetic parameters for M3 are given in Table 2. A number of common features were observed in M3 and OPZ kinetic characteristics. Intersubject variability was high, and there were less than proportional increases in the AUC and Cmax of M3 with increasing...
dose. Secondary concentration peaks were also observed and tended to parallel or slightly lag those observed for OPZ. Examination of the AUCM3:AUC OPZ ratios suggests the possibility of saturable metabolism because the ratios tended to decrease with increasing dose. It is unclear whether this saturable metabolism to M3 is strictly a concentration-dependent phenomenon or is further confounded by an enzyme polymorphism. At the 250 mg/m² dose level, two patients had AUC M3:AUC OPZ ratios of 3.33 and 3.44, whereas two other patients had values of 0.28 and 0.58, suggestive of two divergent populations. The small number of patients (n = 13 at the three higher dose levels) in which these AUC ratios were available prevents a distinction between dose-dependent and genetic polymorphic metabolism of OPZ.

**DISCUSSION**

Chemoprevention of cancer is an emerging therapeutic area that is likely to be based on chronic drug administration, with oral dosage forms as the preferred mode of treatment. In many cases, as with OPZ, a parenteral dosage form is unavailable, thus necessitating pharmacokinetic data to be based solely on results obtained from oral dosing. The reliance on oral-derived phar-M3

**Table 1** Pharmacokinetic parameters of oltipraz in patients receiving a single dose

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>AUC (ng h/ml)</th>
<th>CL/F (liters/h/m²)</th>
<th>V_{ss}/F (liters/m²)</th>
<th>t_{1/2} (h)</th>
<th>t_{max} (h)</th>
<th>C_{max} (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 (5)</td>
<td>2532.7 (2476.6)</td>
<td>81.1 (46.4)</td>
<td>2155.0 (1524.1)</td>
<td>22.7 (0.7)</td>
<td>2.3 (0.7)</td>
<td>157.7 (156.0)</td>
</tr>
<tr>
<td>250 (5)</td>
<td>3119.0 (2441.7)</td>
<td>152.8 (135.9)</td>
<td>2233.4 (1722.3)</td>
<td>10.7 (2.9)</td>
<td>2.4 (0.6)</td>
<td>335.1 (472.3)</td>
</tr>
<tr>
<td>500 (4)</td>
<td>4747.8 (2166.5)</td>
<td>120.2 (44.6)</td>
<td>2610.5 (2092.8)</td>
<td>13.3 (9.4)</td>
<td>2.2 (1.3)</td>
<td>431.3 (353.6)</td>
</tr>
<tr>
<td>1000 (4)</td>
<td>9276.8 (6145.5)</td>
<td>219.6 (251.5)</td>
<td>3506.8 (4071.8)</td>
<td>9.3 (1.8)</td>
<td>1.9 (0.9)</td>
<td>670.5 (9502.8)</td>
</tr>
</tbody>
</table>

* Standard deviation.

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**Fig. 1** Representative chromatograms. The upper chromatogram is that of blank plasma under the conditions described. The lower chromatogram is that of spiked plasma in which OZ represents oltipraz, M2 and M3 the metabolites, and AT(IS) anethole trithione, the internal standard.

**Fig. 2** Concentration-time profiles of OPZ and M3 in a patient receiving a single 1000 mg/m² dose of OPZ.
Table 2 Pharmacokinetic parameters for metabolite M3

<table>
<thead>
<tr>
<th>Dose level (mg/m²)</th>
<th>AUC (ng h/ml)</th>
<th>t½ (h)</th>
<th>tmax (h)</th>
<th>Cmax (ng/ml)</th>
<th>AUC_{M3}/AUC_{OPZ}</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 (5)</td>
<td>3186.8 (1523.6)</td>
<td>22.2</td>
<td>2.5 (0.7)</td>
<td>77.8 (50.7)</td>
<td>a</td>
</tr>
<tr>
<td>250 (5)</td>
<td>3703.8 (2054.8)</td>
<td>17.8</td>
<td>8.6 (15.3)</td>
<td>175.9 (87.7)</td>
<td>0.74 (0.52)</td>
</tr>
<tr>
<td>500 (4)</td>
<td>4522 (1692.3)</td>
<td>22.9</td>
<td>2.7 (0.5)</td>
<td>167.0 (72.7)</td>
<td>0.81 (0.33)</td>
</tr>
<tr>
<td>1000 (4)</td>
<td>5000 (2000)</td>
<td>21.2</td>
<td>2.5 (0.7)</td>
<td>280.2 (140.1)</td>
<td>0.72 (0.36)</td>
</tr>
</tbody>
</table>

*Insufficient concentration data to calculate.

Fig. 3 Apparent clearance (CL/F) of OPZ versus the administered dose (N = 18). Overlapping values were obtained at each dose level. The overlapping apparent clearances at 250 mg/m² are at 71.5 and 71.8 liters/h/m².

macokinetic data can make interpretations difficult because the absolute bioavailability of the drug is unknown. Therefore, interpretation of the paramount pharmacokinetic parameters of clearance and volume of distribution is confounded by the inability to determine the drug’s bioavailability or the fraction of the dose that is absorbed into the systemic circulation. Accordingly, these parameters are designated apparent. These limitations in evaluating orally derived pharmacokinetic data are present in the current OPZ study.

The elimination half-lives of OPZ ranged from a mean of about 9–22 h, with the latter value obtained at the lowest dose level of 125 mg/m². The half-life values at the three higher dose levels were similar and agree with values reported previously (18). A definitive dose dependency in the apparent clearance and volume of distribution of OPZ was not found, yet each of these parameters increased in going from the lowest to highest dose. Again, the limitation of the apparent clearance and volume of distribution parameters makes interpretation of these trends difficult. Increasing apparent clearance and volume of distribution with dose most likely reflect a reduction in the bioavailability of OPZ because the bioavailability term is in the denominator of both expressions defining the apparent clearance (i.e. CL/F) and apparent volume of distribution (i.e., VSS/F). For a compound like OPZ with poor water solubility, a reduction in bioavailability could be attributed to saturable GI solubility. In this case, measurements of OPZ in feces would be needed to verify whether an increased fraction of the dose is excreted in the feces as the dose is increased. The alternative explanation for decreased GI bioavailability, namely saturable GI membrane permeability, seems unlikely for a lipophilic drug such as OPZ that is likely to be transported by simple diffusion. Saturable hepatic metabolism by itself (see below) would not produce the observed changes in apparent clearance of OPZ. Thus, the trends of increased apparent clearance and volume of distribution for OPZ as dose is increased seem most consistent with diminished GI bioavailability due to limited GI solubility.

Use of a new analytical method allowed us to simultaneously measure OPZ and two of its metabolites, M2 and M3. We have previously shown M2 to be active in vitro (12); however, our data show that limited conversion to this metabolite occurs in human subjects. This may further support the investigation of M2 as a chemopreventive drug in its own right. M3 does not induce transcription of phase II genes and thus is an inactive metabolite. Nonetheless, it appears to be a major metabolic product and hence partially accounts for the disposition of OPZ. As with OPZ, large interpatient variability in M3 disposition was observed, and there were less than proportional increases in its AUC and Cmax values with dose. The M3:OPZ AUC ratio decreases with increasing dose, suggestive of a saturable phenomenon most consistent with saturation of the OPZ to M3 pathway. The OPZ AUC decreases less with dose than the M3 AUC and may be partially attributed to other compensatory metabolic pathways. These potential metabolic alterations are superimposed on potential reductions in bioavailability of OPZ. Nonlinear disposition of OPZ due to saturable metabolism has also been suggested by Gupta et al. (19) in a Phase I trial. In that study, OPZ was administered with food, which is presumed to increase absorption, and differs from the fasted state of patients in the current study.

Secondary concentration peaks were observed for OPZ and M3 in numerous patients. Because patients were fasted, and OPZ is poorly soluble in water, erratic and pulsatile absorption could be implicated as the cause of the additional peaks. Another possibility could involve enterohepatic recycling of the M3 glucuronide conjugate, referred to as M13, that has been reported in humans (18). OPZ itself is not a good candidate for biliary secretion or recycling due to its small molecular weight and lipophilicity, which favor reabsorption. Enterohepatic recycling of M13 would entail its initial secretion into bile, cleavage
to M3 by β-glucuronidase in the intestine, followed by reabsorption into the systemic circulation. This process does not account for the secondary OPZ peaks because it is unlikely that OPZ could be regenerated from M13 due to the required intermediary metabolic steps. In a previous Phase I trial in which OPZ was administered with a high fat meal (19), secondary concentration peaks were not observed, indicating the importance of food in modulating its absorption. Thus, the single most plausible explanation for secondary OPZ and M3 concentration peaks is based on pulsatile absorption apparently promoted by the low water solubility of OPZ.

In summary, a Phase I trial of OPZ was completed in which one of its major metabolites has been measured with a new HPLC method. This metabolite reached appreciable plasma concentrations. The disposition of OPZ is characterized by large interpatient variability and potential nonlinearities most likely attributed to saturable metabolism and absorption. OPZ oral absorption is erratic, and administration of OPZ to fasted subjects may lead to secondary concentration peaks. A greater understanding of OPZ pharmacokinetics would be gained through the use of a parental dosage form, enabling determination of bioavailability, and differentiation between absorption and metabolic factors that impact on its disposition.

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