Oltipraz Concentrations in Plasma, Buccal Mucosa Cells, and Lipids: Pharmacological Studies

Nikolay V. Dimitrov, Cheryl Meyer Lecce, Emily R. Tompkins, Elizabeth Seymour, Maurice Bennink, Joseph Gardiner, James Crowell, Ernest Hawk, Mohammed Nashawaty, and James L. Bennett

Abstract

Oltipraz is considered one of the most potent cancer chemoprevention agents, as shown in preclinical studies. Its pharmacological effects in humans have been associated with unusual toxicity affecting the fingers and toes. This study was designed to test intermittent dosing schedules using two dosage levels: 500 mg as a single weekly dose and 200 mg as a biweekly dose, each for 30 days. Fifteen men and women were studied in each dosing group. All were heavy smokers considered to be at high risk for developing lung cancer. Plasma, buccal mucosa cell, and lipoprotein concentrations were measured at different intervals corresponding to the time period when most of the adverse effects occur. No serious toxicities were observed using these doses and schedules. The plasma and buccal mucosa cell concentrations of Oltipraz showed substantial interindividual variations at each sampling. Some subjects had no detectable plasma or buccal mucosal cell Oltipraz concentrations. The distribution of Oltipraz incorporation into the lipid fractions and albumin was changed by the administration of different schedules of Oltipraz. The results of this study suggest that the intermittent dosing is well tolerated and does not result in steady state in plasma or buccal mucosa cells. The variation and lack of detectable Oltipraz concentration in plasma, buccal mucosa cells, and lipids may affect both the toxicity and the pharmacological effects when these doses and schedules are used.

Introduction

During the last two decades, Oltipraz [4-methyl-5-(2-pyrazinyl)-1-2 dithiole-3-thione] has been extensively evaluated as an antischistosomiasis and cancer chemopreventive agent (1–5). However, its use for the treatment of schistosomiasis has been replaced by more efficient agents. In addition, occurrence of phototoxicity during the treatment with Oltipraz has precluded its use in tropical areas. The pharmacokinetics of Oltipraz in humans are well established (3, 4, 6). On the basis of numerous laboratory studies and extensive animal testing, Oltipraz was found to be a promising chemopreventive agent with high overall preventive indices (5). Although Oltipraz exhibits substantive promise based on preclinical testing, it is clinically challenging because of its high lipid solubility, its nonlinear pharmacokinetics, and its differential absorption depending on the fat content of the diet (2, 4, 6). In addition, the recognition of large numbers of metabolites after absorption in animals raises additional questions related to human use (7, 8). Although the dosing and schedules in humans remain a subject for discussion, use of an intermittent schedule in on-going chemoprevention trials appears to be well-tolerated (9, 10). However, monitoring the efficacy of Oltipraz and the observed digital symptoms (e.g., pain) as serious adverse effects in some subjects remains a great concern to all investigators (3–5). The study results reported in this communication are related to Oltipraz digital symptomatology, the pathophysiology of this adverse effect, and pharmacology. It was also designed to investigate the tolerance and effect of two dose levels of Oltipraz, both of which were administered to heavy cigarette smokers considered at high-risk for developing lung cancer.

Materials and Methods

Study Subjects. Thirty subjects consisted of 18 females and 12 males, all heavy smokers (more than 20 pack-years), ranging in age from 32 to 62 years. All subjects had abnormal sputum cytology at baseline. Normal chest X-ray, complete peripheral blood counts, and biochemical profiles were required by the eligibility criteria for participation in the study. Premenopausal women required a negative pregnancy test before entrance into the study and followed appropriate contraceptive measures while participating in the protocol. Before the study, a physician evaluated all subjects eligible to participate. In addition, the eligible subjects were required to sign an appropriate informed consent form approved by both the NCI and the University Committee on Research Involving Human Subjects at Michigan State University.

Drug Supply. Oltipraz was provided by the Chemoprevention Branch, NCI, and produced by the Rhône-Poulenc Company.
Oltipraz in Plasma, Buccal Cells, and Lipids

(Paris, France). The purity of the drug was >99% as determined by high performance liquid chromatography and gas liquid chromatography-mass spectrometry. The drug was supplied in capsules containing 100 and 250 mg of Oltipraz in lactose.

**Dose and Schedule Selection.** On the basis of previous pharmacological trials, doses of 500 mg of Oltipraz once weekly for 30 days and 200 mg twice weekly for 30 days were considered safe and capable of modifying several biochemical markers (9, 10). Because one of the major objectives of this study was to observe changes in the neurovascular systems of the fingers and toes, the collection of blood samples was scheduled to meet that purpose and not the rules of pharmacokinetics and pharmacodynamics. Thus, the blood sample collection does not correspond with the dosing, but to the time of occurrence of eventual finger/toe toxicity considered as a major side effect of Oltipraz treatment.

**Drug Administration.** Subjects ingested Oltipraz p.o. at 7:30 a.m. with 180 ml of whole milk on an empty stomach. Milk was given to provide a uniform environment during the absorption of this lipid-soluble compound. The capsules were ingested in the presence of the drug distributor, assuring 100% compliance with protocol requirements.

**Buccal Mucosa Cell Collection.** A revised method similar to that of Newcomb et al. (11) was used to collect buccal mucosa cells. Subjects were required to rinse their mouths with tap water and then brush each cheek firmly in an up and down motion with a medium-hard toothbrush 20 times. Subjects then rinsed their mouths for 30 s with 20 ml of 0.9% NaCl before depositing the rinse in a 50-ml centrifuge tube. The toothbrush was rinsed in an additional 10 ml of NaCl and then the rinse solution was added to the tube. The cells were concentrated by centrifuging for 15 min at 2500 rpm (272,000 × g) at 20°C. A low acceleration rate and no brake were used. After separation, the lipoprotein fractions were removed and frozen until Oltipraz determination. Extractions were performed as with plasma, except the lipoprotein fraction volumes varied from 0.5 ml to 3.0 ml, and the heptane volumes were adjusted accordingly.

**Sample Collections and Clinical Laboratory Studies.** Blood samples and buccal mucosa cells were collected before entry into the study for baseline values and on days 4, 11, 16, and 30. These are time points when digital toxicity was observed in past studies and does not represent sampling for pharmacokinetic purposes.

Routine peripheral blood counts, including differential counts of leukocytes, biochemical profiles, and lipid measurements, were conducted before entry into the study and after completion.

**Evaluation of Side Effects.** Standard questions regarding nausea, vomiting, change in bowel habits, tingling, numbness, discoloration and/or pain in fingers and toes, and muscle weakness were asked by the drug distributor every day on which the subject was seen (days 4, 11, 16, and 30). Inquiries regarding side effects were also made by telephone on days 5–15, when participants were not seen in the clinic, and on days 37 and 44 (after discontinuation of the treatment on day 30). If any complaint was reported, the subject underwent a physical examination by the physician participating in the study, with vascular and neurological examination for selected subjects with complaints of pain or numbness. Recording of adverse effects was done according to the NCI Common Toxicity Criteria. Complete blood counts, urine analysis, and biochemical profile tests were done at the conclusion of the study.

**Statistical Analysis.** Statistical analyses were conducted to compare responses between two groups: Group 1 subjects, receiving a single dose of 500 mg/week, and Group 2 subjects, receiving 200 mg twice weekly. The analyses were arranged according to the different laboratory studies and are presented in the “Results” section for each study group. Comparison between groups was made by using the Wilcoxon nonparametric or t test as appropriate (14).

**Results**

**Toxicity**

The results for final evaluation of the adverse effects are presented in Table 1. The toxicities according to the NCI Common Toxicity Criteria were mild and presented as grades 1 and 2, as indicated in the Table. Itchy, tingling fingers occurred in two subjects on the weekly schedule and in one subject on the biweekly schedule. Low-grade finger pain was reported in two subjects on the biweekly schedule. However, no anatomical skin changes were observed. These complaints lasted a short time, no longer than 24 h. Subjects underwent immediate vascular and neurological testing, and no abnormal findings were recorded. Low-grade, short-lasting nausea; vomiting; flatulence; diarrhea; and headache were reported in a few cases. Only one subject reported short-lasting blurred vision.

**Plasma Concentrations of Oltipraz—Weekly Dosing**

The mean plasma concentrations of Oltipraz for subjects taking one dose of Oltipraz 500 mg weekly are presented in Fig. 1. As mentioned in “Materials and Methods,” the sample collection for measurement of Oltipraz concentration in the plasma was not designed for pharmacokinetic purposes, but to match eventual occurrences of finger/toe toxicity. In addition, this schedule...
demonstrates that intermittent dosing sustains the presence of Oltipraz in plasma and tissue. By day 4 after the administration of the first dose of Oltipraz (500 mg), the plasma level of Oltipraz was measurable at 3.0 ± 0.65 ng/ml. On day 16, which corresponded to that of plasma Oltipraz concentrations. Eleven subjects from this group showed trace levels or no detectable Oltipraz in the samples of buccal mucosa cells.

**Table 2 and Fig. 2.** Oltipraz concentrations were determined using 10^6 cells. The patterns of buccal cell concentrations for days 4, 11, 16, and 30 were similar to those of the plasma concentrations. The data showed a drop in Oltipraz concentrations on day 16, which corresponded to that of plasma Oltipraz concentrations. Eleven subjects from this group showed trace levels or no detectable Oltipraz in the samples of buccal mucosa cells.

**Buccal Cell Concentrations of Oltipraz—Biweekly Dosing**

The Oltipraz concentrations of buccal mucosa cells for subjects taking Oltipraz 200 mg biweekly were presented in Table 2 and Fig. 2. The pattern of the Oltipraz in buccal mucosa cells was again similar to that of plasma concentrations (Fig. 2). This pattern also resembles the pattern of plasma and buccal cell concentrations observed with the single weekly dose of Oltipraz (500 mg). Eleven subjects from this group showed trace levels or no detectable Oltipraz in the buccal mucosa cells.

**Comparisons Between Groups**

Nonparametric procedures were used to compare each concentration, for a total of eight pairs. These pairs contained only those subjects who had sufficiently measurable Oltipraz concentrations in the buccal mucosa cells. No adjustment was made for multiple comparisons. There was no difference in buccal cell concentrations, but plasma concentrations showed significant differences at day 11 (P = .011) and day 16 (P = .017) only.

**Comparison Within Groups**

Changes from day 4 were computed within each dose group. We used sign tests to assess the significance of these changes. For buccal cell concentrations, none of these changes were significant in either group, with Ps ranging from .23 to 1.0. For plasma, significant changes were noted in the group taking the single weekly dose (500 mg) only from day 4 to day 16. Furthermore, this change and that from day 4 to day 11 passed the formal Shapiro-Wilk test for normality (P = .63 and P = .40, respectively).

**Lipoprotein Distribution**

**Oltipraz Distribution in Lipoproteins—Weekly Dosing.**

Blood samples were obtained on days 11 and 30 from subjects taking a single 500-mg weekly dose of Oltipraz. The concentrations of Oltipraz incorporated into the lipoprotein fractions are presented in Table 3. The percentage of Oltipraz incorporated into the lipoproteins was determined, and the incorporation into the individual lipoprotein fractions and albumin are presented in Table 4. The albumin fraction incorporated 24% of the total plasma concentration of Oltipraz. The rest was incorporated into HDL (29%), LDL (23%), VLDL (13%), and IDL (11%). On day 30, the pattern of distribution changed (Table 4). The albumin fraction incorporated only 4% of the total plasma concentration, which was a substantial decrease compared with day 11 (24%). HDL and LDL were unchanged, but an increase in VLDL incorporation (from 13% to 27%) and IDL incorporation (from 11% to 18%) was observed (Table 4).

**Table 1** Toxocities associated with oltipraz administration

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Dose (mg/wk)</th>
<th>Grade</th>
<th>No. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vomiting</td>
<td>500</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Dizziness</td>
<td>500</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>500</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>500</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>500</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Headache</td>
<td>500</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Itchy/tingling fingers</td>
<td>500</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Shoulder/back pain</td>
<td>500</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>500</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Nausea</td>
<td>200×2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>200×2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>200×2</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Itchy/tingling fingers</td>
<td>200×2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Flatulence</td>
<td>200×2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vomiting</td>
<td>200×2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Fatigue</td>
<td>200×2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Back pain</td>
<td>200×2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Finger pain</td>
<td>200×2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>200×2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Plasma Concentrations of Oltipraz—Biweekly Dosing**

Plasma concentrations of Oltipraz for subjects taking two separate doses of Oltipraz 200 mg biweekly are also presented in Table 2 and Fig. 1. As in the single weekly dose, individual variations existed in the plasma concentrations, with the highest concentrations observed on day 4 after the administration of the first dose. Measurements on days 11 (3 days after the third dose) and 30 (5 days after the last dose) revealed lower concentrations than on day 4. On day 16 (5 days after the fourth dose), only low concentrations, close to zero, were observed. As in the weekly dose schedule, the timing of drug administration and sample collection did not coincide.

**Buccal Cell Concentration of Oltipraz—Weekly Dosing**

The concentration of Oltipraz in buccal mucosa cells for the fifteen subjects taking Oltipraz 500 mg weekly is presented in Table 2 and Fig. 2. Oltipraz concentrations were determined using 10^6 cells. The patterns of buccal cell concentrations for days 4, 11, 16, and 30 were similar to those of the plasma concentrations. The data showed a drop in Oltipraz concentrations on day 16, which corresponded to that of plasma Oltipraz concentrations. Eleven subjects from this group showed trace levels or no detectable Oltipraz in the samples of buccal mucosa cells.
plasma Oltipraz, which was lower than the single-dose schedule (24%). VLDL incorporation (22%) and LDL incorporation (19%) were similar to the single dose schedule; 13% and 23%, respectively. Substantial changes were observed in HDL (11% versus 29%), and IDL (38% versus 11%), for the single-dose schedule. Changes also occurred on day 30 as compared with day 11 (Table 4). The percent of Oltipraz incorporation into the albumin fraction increased from 9% to 15%. In addition, Oltipraz incorporation increased in the VLDL from 22% to 34%. In contrast, Oltipraz incorporation decreased in HDL from 11% to 6%, in LDL from 19% to 13%, and in IDL from 38% to 32%.

When the patterns of both dosing schedules on day 11 are compared (Table 3), some substantial differences in all fractions were seen. Similar differences were observed when the two dosing schedules were compared on Day 30.

The changes in lipoproteins and albumin fractions had no substantial correlation with the Oltipraz plasma concentrations and the concentrations of the buccal mucosa cells. However, the shift in lipoprotein concentrations and the change in the ratio of albumin to lipoprotein concentrations were significant.

Discussion

This clinical study was an extension of previous studies related to the toxicity, pharmacokinetics, and pharmacodynamics of Oltipraz (3, 4, 6, 10). The study population was restricted to heavy smokers, men and women at risk to develop lung cancer. In addition to plasma and mucosa cell Oltipraz concentrations, we have also studied lipoprotein and albumin concentrations, because lipoproteins participate in the transport of Oltipraz (a fat-soluble drug). Measurement of Oltipraz in buccal mucosa cells is important because they represent tissue directly exposed to the tobacco smoke, and the presence of Oltipraz may represent part of the pharmacodynamics of the drug.

The results from this study reveal that weekly administration of higher-dose Oltipraz (500 mg) resulted in the elevation of plasma concentrations. The plasma concentration curve did not show a tendency toward steady state. Six participants showed no presence of plasma Oltipraz, which indicates variation in absorption among this group of individuals. Because p.o. administration compliance was 100%, this finding should be considered as part of the interindividual variations. The erratic pattern of plasma levels is also attributable to a discrep-

Statistical Analysis for Lipid Profiles

Lipid profiles were computed from VLDL, LDL, IDL, HDL, and albumin readings. The measurements are the percentage of total (of all four lipids and albumin fractions) at day 11 and day 30. Note that the within-subject values for these lipid measures will sum to 100%. As expected, there is high correlation between these lipid measurements. Comparisons were made between the two groups of each lipid fraction using nonparametric tests. At day 11, the groups differed on IDL ($P = .025$) and HDL ($P = .04$). At Day 30, the only significant difference was in HDL ($P = .026$).

We computed the difference in each lipid measure from day 11 and day 30. These differences were considerably less skewed (the albumin difference was the only possible exception), and normal distribution theory was used to compare the difference between groups. None of these differences (VLDL, LDL, IDL, and HDL) were statistically significant, with $P$s ranging from .60 to .94. We used a nonparametric test to compare the change in albumin and determined that it was also not significant ($P = .06$).
ancy between dosing and samples collection (Fig. 1). The protocol design required weekly dosing, (500 mg or 200 mg × 2), but the collection of blood took place on days 4, 11, 16, and 30 after the first dosing. Seven subjects showed no detectable Oltipraz. For the biweekly dosing schedule, the second dose was administered on day 4, the third dose on day 8, the fourth dose on day 11, the fifth dose on day 16, the sixth dose on day 18, the seventh dose on day 22, and the final dose on day 25.

The pattern of the concentration curves was different when two 200-mg doses of Oltipraz were given weekly. The plasma concentrations were higher for several subjects compared with previous measurements. Although we have no exact explanation for this pattern, the possibility of a rebound of Oltipraz stored in the adipose tissue (fat-soluble compound) should be considered. This may explain the discrepancy between plasma and buccal mucosal cells as well. The purpose of the design was to provide measurements of Oltipraz concentrations at intervals when most of the severe adverse reactions occur, hence, between 3 and 10 days after the first dosing. However, Jacobson et al. (10) have demonstrated that the pharmacological effects of Oltipraz continue long after the single dosing of 500 mg.

The pattern of the concentration curves was different when two 200-mg doses of Oltipraz were given weekly. The plasma concentrations were higher for several subjects compared with previous measurements. Although we have no exact explanation for this pattern,
samples were also taken on days 4, 11, 16, and 30. This schedule had no equal intervals. There were substantial interindividual responses to the dosing. Nine subjects from this group had no detectable Oltipraz levels at different times during the sample collections. This observation is attributable to the rapid clearance time demonstrated by previous studies (3, 6). The administration of a lower dose may contribute to the absence of plasma Oltipraz as well.

The buccal mucosa cell concentrations for both groups showed patterns similar to those of the plasma concentrations. In the group of subjects taking Oltipraz 500 mg once weekly, 11 participants showed no detectable traces of Oltipraz, which again suggests interindividual variations in absorption and distribution. No correlation between plasma and buccal cell concentrations were found. The difference between the two dosing schedules on days 4, 11, and 16 showing higher concentrations in the buccal mucosa cell group may be attributable to longer tissue retention when two doses are taken. However, on day 30, the buccal mucosa cell concentrations of Oltipraz are similar. We have no explanation for this pattern.

Comparing plasma with buccal mucosa cell concentrations, there are discrepancies between the two dosing groups. On day 11 of the 500-mg group, the plasma concentration is substantially higher compared with the cells (Fig. 1 and Fig. 2). At the same time, the group taking 200 mg biweekly showed lower concentration for plasma compared with the buccal mucosa cell concentrations (Fig. 1 and Fig. 2). Although we cannot compare plasma (ml) with cellular concentrations (10^6 cells), the patterns appear to be quite different. There are substantial interindividual variations in both dosing groups. In addition, the measurements of a good number of samples did not reveal any presence of Oltipraz. This raises the question regarding the pharmacological effect, the steady state of the drug concentration, and the length of time needed to sustain the pharmacological effect. It has been shown that discontinuation of the chemopreventive agent is followed by the occurrence of neoplastic process (15). However, other authors found that this may not be valid for Oltipraz (9).

The toxicity of Oltipraz appears to be less of a problem when it is administered under our study conditions using two intermittent schedules for 30 days. As shown in Table 1 we observed only mild toxicities (grades 1 and 2). Comparing the toxicities with plasma and buccal mucosa concentrations of Oltipraz showed no relationship between the occurrence, dosing, and severity of the adverse events. It appears that the most common adverse effect was flatulence, which occurred in 9 of 15 subjects taking 200 mg of Oltipraz twice weekly. In the group taking higher daily doses of Oltipraz (500 mg, 300 mg), nausea was the prevalent adverse reaction, which occurred in 6 of 15 subjects. Again, these were low-grade events and are classified as mild for the duration of 30 days.

The results from the measurements of Oltipraz incorporation into the lipoprotein fractions and albumin are considered new information. The pattern of concentration and the percentage of the total amount incorporated into the lipoprotein fractions and albumin showed shifting from one compartment to the other (Tables 3 and 4). This also may result in changes of pharmacodynamics. The lipoprotein shifting is difficult to explain. The changes in the albumin fraction could be related to the functional ability of Oltipraz to inhibit formation of aflatoxin-albumin adducts. As demonstrated by Kensler et al., (16) the exposure to carcinogen in the presence of Oltipraz decreases significantly the formation of aflatoxin-albumin adducts (10). This suggests that the presence of Oltipraz or metabolites in the albumin fraction bring the agent in contact with the toxin, which allows an antioxidant response. Although speculation, this possibility should not be ignored. Besides the penetration of the cell membranes of organs such as the lung, liver etc., this lipidsoluble drug could penetrate the central nervous system and peripheral nerves after presentation by lipoprotein. The mechanisms by which Oltipraz is carried by different lipoprotein fractions, is disposed of, or is stored, remains to be investigated if Oltipraz will be considered for long-term administration in chemoprevention trials. Because new analogues are being proposed, their evaluation related to pharmacology should be done on the basis of all studies done with Oltipraz (17). Inhibition of cigarette smoke-related lipophilic DNA adducts by Oltipraz indicates the significance of this drug in the prevention of target tissues (18). Buccal mucosa is directly exposed to cigarette smoke and could store Oltipraz under certain conditions, making it an appropriate target for such a study. The interindividual variations in absorption and tissue deposition of Oltipraz should be addressed when dosing and schedules are considered.

Acknowledgments
The authors greatly acknowledge the valuable work of Sara M. Sertiz in recruiting and scheduling of participants for this trial and Kay Lockwood for her assistance in the manuscript preparation.

References


Oltipraz Concentrations in Plasma, Buccal Mucosa Cells, and Lipids: Pharmacological Studies

Nikolay V. Dimitrov, Cheryl Meyer Leece, Emily R. Tompkins, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/10/3/201

Cited articles
This article cites 13 articles, 6 of which you can access for free at:
http://cebp.aacrjournals.org/content/10/3/201.full.html#ref-list-1

Citing articles
This article has been cited by 2 HighWire-hosted articles. Access the articles at:
/content/10/3/201.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.