Oltipraz Chemoprevention Trial in Qidong, People’s Republic of China: Study Design and Clinical Outcomes


Abstract
In 1995, 234 adults from Qidong, Jiangsu Province, People’s Republic of China, where hepatocellular carcinoma is the leading cause of cancer deaths and exposure to dietary aflatoxins is widespread, were enrolled and followed in a Phase II chemoprevention trial. The goals of the study were to define a dose and schedule of oltipraz for reducing levels of validated aflatoxin biomarkers and to characterize dose-limiting toxicities. Healthy eligible individuals, including those infected with hepatitis B virus, were randomized to receive either 125 mg of oltipraz daily, 500 mg of oltipraz weekly, or placebo. Blood and urine specimens were collected to monitor toxicities and evaluate biomarkers over the 8-week intervention period and subsequent 8-week follow-up period. Unique trial aspects included a synchronous follow-up schedule, daily observed administration of all medications, timely international data transfer, and use of biomarkers as outcomes. One hundred thirty-two participants took their medications without interruptions, approximately 77% contributed all nine urine samples, and 78% contributed all seven blood samples. Fifty-one participants (21.8%) reported clinical adverse events. An extremity syndrome, developing soon after the start of treatment, was the only event that occurred more frequently (P = 0.002) among the active groups (18.4 and 14.1% of the daily 125 and weekly 500 mg arms, respectively) compared with placebo (2.5%). The oltipraz arms did not differ in symptom type or severity, and there were no indications of exacerbated drug intolerance among the few participants infected with hepatitis B virus. The good compliance with an intense follow-up schedule shows that chemoprevention trials with biomarker end points may be conducted in such populations.

Introduction
HCC3 is one of the most common cancers in China and sub-Saharan Africa and results in an at least 250,000 deaths annually. HCC is the leading cause of cancer death in Qidong City, Jiangsu Province, People’s Republic of China, and accounts for up to 10% of all adult deaths in some of the rural townships outside the city (1, 2). Observational studies conducted in this region of China have shown that chronic infection with HBV is an important risk factor (3, 4), consistent with other evidence for a causal relationship between HBV and HCC (5). However, although the percentage of individuals infected with HBV is constant throughout Jiangsu Province, the incidence of HCC increases more than 10-fold over a 100-km west-east gradient near the mouth of the Yangtze River (2). It has been postulated that exposure to aflatoxins in the diet and algal toxins in the drinking water also contributes to the extraordinarily high risk of HCC in Qidong City (1, 2, 6). Aflatoxins are potent hepatocarcinogens and are consistent contaminants of the food supply in this area, particularly in corn, peanuts, soya sauce, and fermented soy beans. A multiplicative interaction between HBV and aflatoxins for risk of HCC has been demonstrated using a nested case-control study in Shanghai (3, 4). In a recent longitudinal survey of 120 residents of Daxin Township, Qidong City, more than 95% of the participants tested positive for serum aflatoxin albumin adducts throughout the year (7). Furthermore, results from molecular studies also have suggested a role for aflatoxin in the etiology of HCC. Characterization of the mutational spectra in the p53 tumor suppressor gene in HCC from Qidong demonstrated a high frequency (>50%) of AGG→AGT transversion mutations on the noncoding strand at codon 249 (8). These mutations are not observed in liver cancers from low aflatoxin exposure regions of China. Consistent with these findings, exposure of human liver cell lines to...
Oltipraz Chemoprevention

Strategies for the primary prevention of HCC in Qidong City include HBV vaccination programs, improved water quality, and diminished consumption of fungal contaminated staple grains. However, to break the cycle that begins with HBV infection at birth, universal vaccination must be carried out for several generations. Thus, the desired effect of reducing HCC may take a long time to develop. Moreover, the extent of aflatoxin contamination in foods is a function of the ecology of molds and is not completely preventable. Secondary prevention programs, such as chemoprevention, may be useful in this high-risk setting. Oltipraz, a substituted 1,2-dithiole-3-thione originally developed for the treatment of schistosomiasis, has been shown to produce complete protection against HCC at low dietary concentrations when fed to rats throughout the period of intervention with oltipraz provides substantial protection against hepatic tumorigenesis (12, 13). Chemoprevention in these animal models is reflected in lowered levels of several aflatoxin biomarkers [i.e., aflatoxin-N7-guanine in urine (10) and aflatoxin-albunmin adducts in serum (14)]. Phase I clinical trials with oltipraz indicate that plasma concentrations of the drug, equivalent to those observed in the rodent chemoprevention studies, are easily obtained in humans without significant adverse effects (15-17). On the basis of these collective clinical and experimental findings, a Phase II chemoprevention trial with oltipraz was recently conducted in Qidong. This randomized clinical trial had two major objectives: (a) to preliminarily assess the efficacy of oltipraz by examining modulation in the levels of several biomarkers of aflatoxin in the blood and urine of the study participants; and (b) to characterize in more detail the range of dose-limiting toxicities in a potential target population, including individuals infected with HBV. Elements of the study design and results describing the range of dose-limiting toxicities are the subject of this report.

Materials and Methods

Overall Design and Structure. The Oltipraz Chemoprevention Trial was a Phase II randomized clinical trial designed to evaluate weekly and daily administrations of oltipraz for reducing urinary and/or serum aflatoxin biomarkers compared with placebo. The second objective was to confirm the maximum safe dose of oltipraz after chronic exposures. A synopsis of the study design is provided in Table 1. The goal was to randomize 240 adults in good general health, without any history of major chronic illnesses and with high serum aflatoxin adduct levels at baseline, into three intervention arms: arm A, placebo; arm B, 125 mg of oltipraz administered daily; and arm C, 500 mg of oltipraz administered weekly. Two identical capsules containing either placebo or active drug (125 or 250 mg of oltipraz) were administered daily for 8 weeks. In practice, each daily administration in intervention arm B consisted of one capsule with 125 mg of oltipraz and one placebo capsule. In intervention arm C, individuals received 500 mg of oltipraz (two 250-mg oltipraz capsules) on the first day of each weekly cycle, followed by two placebo capsules for each of the 6 subsequent days. All participants enrolled in the trial, and the village doctors were provided with calendar posters that indicated the days of pill administration and specimen collection. In addition, participants were offered remuneration for their participation, with final payment provided at the last visit.

Table 1 Oltipraz Chemoprevention Trial design synopsis

<table>
<thead>
<tr>
<th>Type of trial</th>
<th>Sample size</th>
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<tbody>
<tr>
<td>Prophylactic</td>
<td>234 (1006 screened)</td>
</tr>
<tr>
<td>Centers</td>
<td>Treatment groups (two pills administered daily)</td>
</tr>
<tr>
<td>Clinic</td>
<td>Placebo control</td>
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<tr>
<td>Daxin Medical Clinic, Jiangsu Province, People’s Republic of China</td>
<td>125 mg of oltipraz administered daily</td>
</tr>
<tr>
<td>Laboratories</td>
<td>500 mg of oltipraz administered weekly</td>
</tr>
<tr>
<td>Qidong Liver Cancer Institute, Jiangsu Province, People’s Republic of China</td>
<td>Treatment assignment</td>
</tr>
<tr>
<td>Shanghai Cancer Institute, Shanghai, People’s Republic of China</td>
<td>Random by DCC</td>
</tr>
<tr>
<td>Johns Hopkins University, School of Public Health, Baltimore, Maryland</td>
<td>Blocks of size 30</td>
</tr>
<tr>
<td>Hagerstown Medical Laboratory, Hagerstown, Maryland</td>
<td>Stratified by hepatitis B status</td>
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<tr>
<td>DCC</td>
<td>Level of treatment masking</td>
</tr>
<tr>
<td>Johns Hopkins University, School of Public Health, Baltimore, Maryland</td>
<td>Participants and data collectors were not informed of treatment assignment</td>
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Length of treatment intervention
- 8 weeks (July 9 through September 2, 1995)
- 8 weeks (September 3 through November 2, 1995)

Data collection schedule (Fig. 2)
- 2 baseline visits, biweekly urine (Tuesday through Thursday), first day blood (cycles 1, 3, 5, 9, 13, 15, and 17), weekly symptom interview, physical exam

Outcomes of interest
- Change in serum aflatoxin biomarkers
- Change in urinary aflatoxin biomarkers
- Change in urinary mutagenesis
- Toxicities

Strategies for primary prevention of HCC in Qidong City include HBV vaccination programs, improved water quality, and diminished consumption of fungal contaminated staple grains. However, to break the cycle that begins with HBV infection at birth, universal vaccination must be carried out for several generations. Thus, the desired effect of reducing HCC may take a long time to develop. Moreover, the extent of aflatoxin contamination in foods is a function of the ecology of molds and is not completely preventable. Secondary prevention programs, such as chemoprevention, may be useful in this high-risk setting. Oltipraz, a substituted 1,2-dithiole-3-thione originally developed for the treatment of schistosomiasis, has been shown to produce complete protection against HCC at low dietary concentrations when fed to rats throughout the period of aflatoxin exposure (10). Oltipraz enhances the metabolic elimination of aflatoxin, in part through the induction of protective detoxication enzymes (11). Recent animal studies have demonstrated that either intermittent dosing or a delayed, transient intervention with oltipraz provides substantial protection against hepatic tumorigenesis (12, 13). Chemoprevention in these animal models is reflected in lowered levels of several aflatoxin biomarkers [i.e., aflatoxin-N7-guanine in urine (10) and aflatoxin-albumin adducts in serum (14)].
chosen on the basis of practical and mechanistic considerations. The daily dose of 125 mg of oltipraz (arm B) reflected the maximum safely tolerated dose of oltipraz from a recent Phase I chemoprevention study of 6 months duration (16, 17). Results of pharmacokinetic studies indicated that peak plasma concentrations of ~1 \mu M oltipraz occurred at this dose, whereas administration of 500 mg produced peak plasma concentrations of 20 \mu M (17). Studies in rodent and human cells in culture indicated that this latter concentration is sufficient to double the activity of a number of carcinogen detoxication enzymes (18). Increases in glutathione levels and activities of glutathione transferases have been noted in lymphocytes obtained from individuals receiving a single, 125-mg dose of oltipraz (17). Although oltipraz has a short plasma half-life, it has an extended pharmacodynamic action reflecting protracted effects on gene expression (12). Thus, the prolonged induction of carcinogen detoxication enzymes by oltipraz, coupled with a lack of evidence suggesting that toxicity increases with accumulated dose (16), led to the evaluation of a weekly dose of 500 mg of oltipraz for arm C. If its efficacy is similar to that observed with daily dosing, this dosing schedule (1 day) should be more tolerated and therefore more acceptable in the population. Observations in animal models also have indicated that intermittent dosing with oltipraz was similar to daily dosing for inhibiting aflatoxin-induced hepatic tumorigenesis (12).

**Sample Size Rationale.** The sample size determination of 240, with 80 in each arm, was based on either intervention reducing aflatoxin-N'-guanine in urine and aflatoxin-albumin adducts in serum by at least 25% when compared with placebo. Using 0.5 as the correlation between biomarker measurements determined three or four times in each of the two phases of the study (intervention and postintervention follow-up), and a 0.75 coefficient of variation (i.e., SD of biomarker/mean level), this sample size provides a 92.3% power to detect such differences. The power computation considered the study as two separate periods (i.e., during and after intervention with oltipraz), to conservatively reflect potentially different effects.

**Recruitment and Screening of Participants.** Study participants were recruited from Daxin Township, Qidong City, Jiangsu Province, People’s Republic of China. Daxin is a rural farming community of approximately 40,000 residents and is located at the mouth of the Yangtze River, 15 km southeast of Qidong City. We enlisted the assistance of the village doctors from four brigades; each brigade contains approximately 1500–2000 people. The village doctors identified potentially eligible residents and asked for volunteers to be screened for the trial. One thousand and six individuals from four villages were screened at the Daxin Medical Clinic within 1 week (May 28 to June 3, 1995). Signed, informed consent was obtained from all participants in accordance with institutional and federal guidelines of the People’s Republic of China and the United States. A medical history, physical examination, liver ultrasound, EKG, and routine hematological, hepatic, and renal function tests were used to screen the individuals, ages 25 to 65 years, at the first baseline visit.

One aliquot of the serum collected during screening was used for determining the ALT level (Beckman Co., Palo Alto, CA), hepatitis B surface antigen status using the AUSRIA II kit (Abbott Laboratories, North Chicago, IL), and \( \alpha \)-fetoprotein status using RIA (Hongqiao Medical Reagent Laboratory, Shanghai, People’s Republic of China) at the Qidong Liver Cancer Institute. The remaining aliquots were frozen at -80°C. For determining eligibility, levels of aflatoxin-albumin adducts were measured in another serum aliquot at the Shanghai Cancer Institute using the method of Wang et al. (7) for sample preparation and RIA. A third aliquot was sent to Baltimore for transfer to a commercial laboratory (Hagerstown Medical Laboratory, Inc., Hagerstown, MD) for measuring blood chemistries (M6: sodium, potassium, bicarbonate, BUN, creatinine, and glucose; M12: albumin, total protein, and total direct bilirubin, ALT, AST, \( \gamma \)-GT, alkaline phosphatase, cholesterol, calcium, phosphorus, magnesium, uric acid, and lactate dehydrogenase). A chemstrip urinalysis was performed using the urine sample obtained at the time of blood draw during the screening period. \( \beta \)-human chorionic gonadotropin was determined from a freshly voided morning urine sample from all women at the time of enrollment.

Individuals were excluded based on an abnormal physical examination, history of a chronic disease (heart disease, kidney disease, liver disease, or cancer, with the exception of nonmelanoma skin cancer), \( \alpha \)-fetoprotein positivity, abnormal liver scan or EKG, abnormal urinalysis, low CBC values (hemoglobin, WBCs, and platelets), abnormal blood chemistry values (urea nitrogen, creatinine, total bilirubin, total protein, albumin, abnormal \( \gamma \)-GT, ALT, AST, alkaline phosphatase, and triglycerides), and outlying aflatoxin-albumin adducts. Women who were pregnant (reported or positive \( \beta \)-human chorionic gonadotropin) or who were lactating also were excluded. Of the 1006 screened individuals, 628 were excluded by at least one criterion from the initial physical examination or clinical laboratory analyses; primary reasons included indications of liver abnormalities (\( n = 368 \)), renal abnormalities (\( n = 204 \)), abnormal hematology (\( n = 182 \)), and cardiovascular problems (\( n = 181 \)). Liver abnormalities included alkaline phosphatase levels >108 IU/liter (\( n = 174 \)), total bilirubin <0.3 mg/dl or >1.3 mg/dl (\( n = 110 \)), abnormal liver function tests (ALT, >55 IU/liter; AST, >45 IU/liter; or ALT, >40 µl (\( n = 78 \))), abnormal \( \beta \)-scan (\( n = 72 \)), liver findings on the physical examination (\( n = 24 \)), and positive \( \alpha \)-fetoprotein (\( n = 14 \)). Renal abnormalities consisted of an abnormal urinalysis (\( n = 128 \)), BUN <6 mg/dl or >23 mg/dl (\( n = 83 \)), or creatinine levels <0.4 mg/dl or >1.12 mg/dl (\( n = 7 \)). Abnormal hematology consisted of platelets <75,000/mm³ (\( n = 108 \)), hemoglobin <11 mg/dl for men and <10.5 mg/dl for women (\( n = 50 \)), or WBCs <3500/mm³ (\( n = 48 \)). Cardiovascular problems included abnormal EKGs (\( n = 123 \)), diastolic blood pressure >90 mmHg or systolic blood pressure >160 mmHg (\( n = 47 \)), abnormal heart findings on physical examination (\( n = 44 \)), or triglycerides >300 mg/dl (\( n = 35 \)). Individuals may have been excluded for more than one reason within a category and between categories. For example, among the 368 people with some liver abnormality, 82 also presented with renal abnormalities, 99 had at least one cardiovascular problem noted, and 22 had findings in all three categories. Among individuals with platelets or hemoglobin levels in the low acceptable range (lower limit to 10% of the limit), only those who were HBsAg negative continued to be eligible, resulting in the exclusion of two additional HBsAg-positive people.

Of the 378 people remaining eligible, 362 had their serum tested for aflatoxin-albumin adducts. Among these individuals, 344 had aflatoxin-albumin adduct values ranging from 1.25 to 10 pmol/mg. These individuals were invited to a town meeting, at which the investigators described the study protocol. A total of 240 agreed to participate; the main reason for declining to participate among the other 98 people was the inability to commit to being in the area for the duration of the trial.

Two hundred thirty-three participants actually reported to the Daxin Medical Clinic on the first day of the study (July 9, 1995), where they completed another physical examination and...
provided blood and urine samples. Urine pregnancy tests at this baseline visit were negative. All study participants remained eligible as determined on site, were given their randomized identification number, and received their first dose of study drug at the clinic. One additional participant, included in the randomization scheme, missed the baseline visit but was allowed to participate starting with cycle 3.

Randomization, Assignment, and Administration of Intervention. After receipt of the 240 screening identification codes of the eligible individuals who were willing to participate and who provided informed written consent for continued participation, the DCC located at Johns Hopkins University School of Public Health, Baltimore, MD assigned them to one of three intervention arms using a fixed randomization scheme. Equal allocation ratios were used, generating 100 randomization assignments to each of the three intervention groups. The randomization block size was 30, which resulted in 10 blocks, ensuring balanced numbers in each intervention arm after every 30 participants. The drug samples assigned the highest 30 numbers were used for drug stability studies performed throughout the trial for quality control by Ogden BioServices Corp., a subcontractor to the Chemoprevention Branch of the National Cancer Institute. The remaining study numbers were stratified by HBsAg status.

Study drugs were packaged in blister packs in weekly units, labeled with a participant’s study identification number, week number, name, and name of the assigned village doctor, and stored in a locked, air-conditioned room at the Qidong Liver Cancer Institute until distribution to the village doctors. To ensure that the drug was taken, village doctors visited their roster of participants daily to dispense the capsules and observe ingestion. Generally, drug was distributed in the morning shortly after breakfast. Used blister packs were returned by the village doctors at the end of each week, at which time the next weekly allotment was provided to them. Compliance was determined by pill counts of the returned packages, performed initially at the clinic and repeated at the DCC when the intervention phase was completed. For this report, days when only one of the two pills was administered were considered as days with missed or incomplete drug usage. This occurred only four times, three times among those receiving placebo and once among those receiving 500 mg of oltipraz weekly. This latter event occurred on a weekday when placebo capsules, rather than active drug, were administered. Eight blister packs were missing when performing the pill counts; they were reported as empty and were inadvertently thrown away by a village doctor’s spouse.

Follow-Up Data Collection Schedule. Urine and blood samples, collected throughout the 8-week intervention and subsequent 8-week postintervention follow-up (Fig. 1), provided the basis for monitoring toxicities and measuring aflatoxin biomarkers. Three consecutive (Monday, Tuesday, and Wednesday) overnight 12-h urine collections were obtained on Tuesday, Wednesday, and Thursday mornings every 2 weeks. A chemstrip analysis was performed using 15 ml from the first sample of each biweekly collection. Blood samples were collected at baseline and on the first day of cycles 3, 5, 9, 13, 15, and 17. Aliquots of urine and serum were stored at −80°C. Portions of each sample were shipped frozen by airfreight to Baltimore. Serum samples from weeks 1, 5, 9, and 17 were immediately transferred to the Hagerstown laboratory for blood chemistry analyses as specified above. All analyses were completed within 96 h of blood collection. ALT determinations and CBC measurements also were done on fresh serum samples from each collection at the Qidong Liver Cancer Institute. Whereas the CBC determinations during screening were made using the residual blood in the tubing of the butterfly needles, samples for these analyses were collected in 2-ml purple-top Vacutainers (Becton Dickinson) in weeks 9 and 17.

Evaluation of Toxicity. Laboratory measurements and self-reported symptoms were used to assess potential toxicities. The grade 1 (mild, defined as slightly bothersome and which can be relieved with symptomatic treatment), 2 (moderate, defined as bothersome and interferes with activities; only partially relieved with symptomatic treatment), and 3 (severe, defined as preventing regular activities; not relieved with symptomatic treatment) criteria of Tangrea et al. (19) were used to record a participant’s subjective assessment of severity. In addition to the daily visits by the village doctors, a symptom report was completed weekly on interviews with each participant, to standardize the data collection and log minor complaints that otherwise may have been missed. The questionnaire included a symptom checklist and an open-ended question to elicit any other complaints. Full medical histories and physical examinations were performed at weeks 4, 8, and 17. The study drug was discontinued when symptoms or laboratory evidence of grade 2 or grade 3 toxicity were encountered. Participants were followed until symptoms resolved. In a limited number of cases, with the mutual consent of the participant, the local physician, and the on-site physician from Johns Hopkins University, individuals who stopped drug briefly for symptoms were rechallenged with the assigned study drug at the same dosage. Individuals with abnormal laboratory values were not rechallenged. Mild abnormalities in hepatic transaminase activities (greater than the upper normal limit) were sufficient cause for discontinuation in individuals who were HBsAg positive. The clinical director at the Qidong Liver Cancer Institute completed an adverse event form for every reported toxicity, including an assessment of its relationship to the use of the study medication. Laboratory evidence of grade 2 or grade 3 toxicity noted from the results of the blood chemistries performed at the Hagerstown Medical Laboratory were immediately reviewed by the study investigator at Johns Hopkins University and forwarded to the Qidong Liver Cancer Institute for follow-up. Listings summarizing the history of laboratory data were generated by the DCC for each participant noted with an adverse event and forwarded concomitantly with the notice of a laboratory adverse event to the on-site investigative team.

Data Management and Statistical Analyses. All data collection forms used in the study were bilingual. Information recorded on the standardized color-coded data entry forms in the People’s Republic of China during the drug intervention phase were entered on-site into preformatted data bases by the principal investigator (T. W. K.) and electronically transmitted to the DCC. Data collected during the postintervention follow-up period were entered at the DCC using similar, preformatted data bases and independent double entry. Some editing procedures were programmed into this data entry system. Data editing performed by the DCC included range checking on each variable, checking the match of key variables used to identify participants and comparing laboratory values with reports of adverse events and daily pill counts for consistency of information. The Hagerstown laboratory electronically sent compressed files with the blood chemistry data to the DCC within 24 h of generation. These data were checked for missing information and outliers (used for both quality control and confirmation of values indicating grade 2 or grade 3 toxicities), and were compared with the other data collected during the same
week to confirm that missing records were consistent with known attrition. Summary reports of all laboratory data collected during the follow-up were generated by the DCC and shipped to Qidong at intervals coinciding with receipt of the blood chemistry data from Hagerstown.

In this report, the distribution of baseline values, study compliance (attrition and adherence to drug administration and specimen collection protocols), and occurrences of toxicities are described overall and by intervention group. Fisher’s exact tests were used to compare the intervention groups for differences in characteristics that have categorical responses, and t tests were used to determine whether mean values of continuously distributed variables differed across intervention groups. Kaplan-Meier survival estimation was used to examine the development of adverse events; participants were classified with a clinical adverse event at the time of initial occurrence, regardless of whether they were then rechallenged with drug. Time-to-event was defined as the time from July 9, 1995 (trial onset) to event or, if no event, to last contact during the intervention period ending September 3, 1995, because clinical events did not occur in the period after intervention. Log-rank statistics were used to test the difference in developing adverse events by treatment (20).

Results
Enrollment and Comparability of Intervention Arms. A total of 234 individuals was randomized to the three intervention arms and administered drug, representing 97.5% of the recruitment goal. The intervention groups were similar for age, gender, HBsAg status, and baseline aflatoxin values (Table 2). As shown in this table, these distributions also are representative of the screened population. Of the 89 individuals (8.8% of the 1006) determined to be HBsAg positive during screening, 3, 4, and 4 were enrolled in the three intervention arms. The intervention groups also did not differ for levels of WBCs, hemoglobin and platelet counts, renal function results, or liver function results. Blood chemistry profiles and results from urinalyses were also similar across groups. There was little to no variation in the parameters obtained at baseline compared with these screening values.

Compliance and Data Collection Completeness. Adherence to study protocol was relatively good despite differences between the active drug arms compared with the placebo group. A total of 196 participants (83.3%) was administered drug through week 8 of the intervention: 92.5, 75.0, and 82.1% of the placebo, 125-mg arm, and 500-mg arm, respectively. Although only 132 participants took their drug for the entire period without interruptions, distributed as 71, 47, and 50% of the placebo, 125-mg arm, and 500-mg arm, respectively, 192 (93, 74, and 80% of each arm) took their drug for at least 44 days (80% of the intervention period). For those who intermittently missed taking drug, there were no patterns specific to any of the intervention groups. Thursdays and Fridays of the cycle accounted for 42–52% of all the missed days across interventions. For the group that was administered 500 mg of oltipraz weekly, active drug was administered on Sunday. If Sunday disproportionately accounted for missed drug days in this group, not only might analyses of the efficacy be affected due to misclassification, but it may indicate underreporting of drug-related symptoms. There were 23 participants in this arm who did not withdraw from the trial during the intervention period but were intermittent drug users; these do not count individuals who stopped drug because of experiencing an adverse event, and stopped again before the end of intervention when rechallenged. Sunday accounted for only 2 (2.9%) of the 68 total days on which these participants did not take drug.

As delineated in the data collection schedule (Fig. 1), a total of nine urine samples and seven blood samples was to be collected from the participants over the course of the trial. Adherence to this protocol was good, with approximately 77% contributing all urine samples (85, 69.7, and 75.6% of the placebo, 125-mg group, and 500-mg group, respectively) and 78% contributing all blood samples (88.8, 69.7, and 75.6% of the placebo, 125-mg group, and 500-mg group, respectively). The differences between the placebo group and the “active drug” groups reflect differences in drug-related attrition (Fig. 2). Specimen collection and processing were performed on the same cycle days for all participants. The distributional differences by intervention are consistent with the observed differences in attrition among the three groups.

A total of 44 participants withdrew from the study before week 17; 39 left during the drug intervention phase, and 5 left during the postintervention follow-up. The attrition by intervention group was 8 in the placebo arm, 20 in the 125-mg arm,
and 16 in the 500-mg arm (Fig. 2). Twenty-eight (63.6%) of the withdrawals during intervention were determined in the field to be related to drug and were distributed as 2, 16, and 10 in the placebo, 125-mg arm, and 500-mg arm, respectively. The two primary reasons for withdrawal among the other 11 participants were that they were "tired of participation" and that the participants "moved from Qidong City." The attrition differed ($P = 0.002$, $\chi^2$ test) by village, with 23.1, 31.1, 4.8, and 17.2% dropping out across the four villages. When controlling for treatment assignment, a difference by village still existed, with one of the villages having significantly lower attrition than the others.

**Adverse Events and Toxicities.** A total of 51 individuals reported clinical adverse events while under intervention. Overall, 11.3% of the placebo group reported an adverse event; this proportion was significantly ($P < 0.05$) lower than the clinical events occurring among the 125-mg arm (29.0%) or the 500-mg arm (25.6%). Time to the initial event differed between the placebo and treatment groups, but not between treatment groups, as shown in the Kaplan-Meier curves in Figure 2. The majority of adverse clinical events occurred shortly after initiating treatment; 68.2 and 75.0% of individuals with events in the 125-mg arm and 500-mg arm, respectively, developed their symptoms in the first week.

The distribution of symptoms by type and grade for each intervention group is presented in Table 3. These conditions were similar to those reported in earlier studies of oltipraz toxicities (16), and all were resolved before study termination. Overall, there were no statistically significant ($P > 0.05$) differences in symptom type or grade between the two oltipraz dosing arms. A syndrome involving numbness, tingling, and sometimes pain in the extremities was the most frequently reported symptom (11.5% of all participants). It typically involved the thumbs and forefingers, although involvement of the other fingers and toes was noted in more severe cases. The time to first occurrence ranged from a few hours to 1 month (33 days) after taking drug. This syndrome was the only adverse event significantly ($P = 0.002$, Fisher's exact test for $3 \times 2$ contingency table) more reported in the 125-mg (18.4%) and 500-mg (14.1%) oltipraz arms compared with the placebo group (2.5%). As shown at the bottom of Figure 2, there was a cross-over in time to this event between the active drug arms. Although the events occurred soon after initiating treatment, in the 500-mg arm, most occurred within days of initiating treatment, whereas the occurrence was more uniform among those receiving 125 mg of oltipraz daily. In all cases, symptoms resolved within 1 week after discontinuation of the study drug. One participant, upon resolution of his extremity pain with the use of an anti-inflammatory during the first week, chose to be rechallenged with drug the second week and had another occurrence. He again wished to remain active upon resolution, and although he was observed to have a minor recurrence, he stayed active for the remainder of the trial. Among the 10 participants who were rechallenged with drug after experiencing an adverse event, only 3 developed the same adverse event subsequently; 2 had episodic events of the syndrome described above, and 1 had repeated bouts of nausea. None of the other seven experienced any adverse events subsequent to being rechallenged. The severity (using the highest grade noted when more than one event was reported) distribution of the adverse event for which the participants first discontinued drug and then were rechallenged was 3 with grade 1, 6 with grade 2, and 1 with grade 3.

Only slight gender differences in reactions were noted. More women than men reported nausea and other gastrointestinal problems, but this difference was observed across all intervention arms. The association of the extremity syndrome with active drug persisted for both genders, but the pattern differed. More men taking the weekly dose reported this syndrome; however, it was more frequently reported among women taking the daily dose of oltipraz. Stratification into body mass index (body mass index = weight/height$^2$) tertiles was done to examine whether relative leanness affected the development or awareness of such conditions. Although differences in reporting the extremity syndrome by intervention group were similar, a trend for increasing occurrence with decreasing body mass was observed; overall, the syndrome occurred in 6.3, 12.7, and 15.8% of those in body mass index categories $>24.22$, 21.76–24.22, and $<21.76$, respectively. There were no consistent trends in reporting symptoms by type of oltipraz dosing. Results from multivariate analyses are not presented because they do not provide any additional information; the trends were consistent, and none of the baseline hematological parameters or liver function tests significantly predicted any event.

Forty-nine participants exhibited elevated liver function tests during the trial: 19 in the placebo arm, 18 among those receiving 125 mg of oltipraz daily, and 12 in the 500-mg oltipraz arm. Three individuals discontinued drug; two of these individuals were in the 125-mg arm, and one was in the placebo group. Only one of these individuals was HBsAg positive. By the end of the study, liver function test values for 18 of the 49 participants had not returned to baseline levels. This occurrence was similar across all treatment arms.
Discussion
There were several unique methodological aspects of this Phase II trial. (a) Enrollment and follow-up of the participants were synchronous. (b) Pills were administered daily, and ingestion was observed. (c) International transfer of data and responses were timely (within days of collection). (d) Biomarkers were used as eligibility criteria as well as for outcomes. Although a staggered data collection was considered, the synchronized approach was more efficient for the collection, processing, and shipping of specimens. In addition, the availability of personnel in the People’s Republic of China permitted the intensive follow-up schedule and method of administering medication. The synchronous follow-up schedule and timely data transfer also were facilitated by having the principal investigator on-site throughout the screening and intervention process. His presence enhanced local understanding of and adherence to the protocol. Logistical problems that did arise were resolved quickly because of the central understanding of the situation and the implications on the study. The ability to screen and assign treatment so quickly was aided by the immediate data entry and transfer by the investigator. Having a single point person for all clarifications and adverse event notifications increased the efficient communication. Much electronic communication (telephone, facsimile, and e-mail) transpired during the screening and intervention phases of the trial.

Other technical features that helped to standardize data collection and processing included supplying bilingual data collection forms and manual of operations and providing coded (IDs, week numbers, dates, etc.) labels to use on the forms and specimens. The processing of the specimens and data was quick; within 24 h of receipt, the laboratory sent results to the DCC, and within the next 48 h, these data were put into the data base, cleaned, and summarized. Reports with these data were then sent to the clinicians in the People’s Republic of China. The timely turnaround of data not only served to notify investigators of laboratory adverse events, but also provided useful information for the clinical care of the participants. This service fostered interest of the investigators and indirectly enhanced continued follow-up of the participants.

Attrition and compliance are measures of the success of a trial and provide information concerning drug tolerance, internal validity of results, and whether similar methods may be used in other trials. The willingness of an individual to remain active in a trial depends on the amount of inconvenience associated with the trial and often involves a personal trade-off between perceived benefits (e.g., health maintenance, potential health gains from treatment) and risks (e.g., discomfort from study procedures, side effects associated with treatment; Ref. 21). Compliance with the number of blood draws was a concern of the central understanding of the situation and the implications on the study. The ability to screen and assign treatment so quickly was aided by the immediate data entry and transfer by the investigator. Having a single point person for all clarifications and adverse event notifications increased the efficient communication. Much electronic communication (telephone, facsimile, and e-mail) transpired during the screening and intervention phases of the trial.

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before study onset because there is a reluctance to donate blood in rural China. However, 78% complied by contributing all seven blood samples in trial, belying this concern. One facilitating factor may have been the close interaction between health care providers and the population. A tiered remuneration schedule also was used to encourage the participants to remain active, despite the intensive follow-up schedule. The relatively short duration of the trial (16 weeks), made possible by the use of biomarker end points, was another key factor in sustaining participation. Chemoprevention trials in which the observation of cancer incidence or death is used as the outcome typically require large sample sizes and long durations (22), increasing the probability of attrition.

In this study, attrition mainly reflected the occurrence of adverse events, and was very inconsistent across the four villages. In the village with the highest dropout rate, the village leader was an active proponent of the trial until developing paresthesia and pain. This village also disproportionately accounted for 43.6% of all of the cases that developed the "extremity syndrome." Some of these cases occurred among those who received placebo, suggesting a psychological component. By contrast, a significantly decreased (6-fold lower) dropout rate was observed in another village, without any apparent explanation. Clearly, the conduct of clinical studies in confined geographical domains (i.e., adjacent villages), with its attendant psychosocial manifestations, exacerbates the potential for over- or underreporting of side effects.

Nonetheless, the type and timing of adverse events were consistent with those reported in previous studies of oltipraz use (15, 16, 23–26). In the early reports evaluating the use of oltipraz as an antichistosomal agent, nausea, vomiting, abdominal pain, headaches, and paresthesias were the primary side effects reported (23–25). Somnolence (25), dizziness and headache (24), and blurred vision (26) were reported less commonly. All of these symptoms, which were transient, occurred within 24 h of initial dosing. The extremity syndrome observed in the present study is reflective of the extremity paresthesia (23), fingertip pain (26), and finger pain/numbness (15) described previously in patients receiving oltipraz. In the present study, the syndrome was manifest principally as numbness, tingling, and sometimes pain in the thumbs and forefingers. In some cases, these symptoms extended to the remaining fingers and to the toes. Such a pattern might suggest a neurological basis for the effects. However, two individuals presenting with grade 2 symptoms developed small subungual hemorrhages beneath the thumbnails. These thumbnail manifestations were observed in one individual receiving chronic treatment (1.5 mg/kg/day) with oltipraz during the Phase I trials (15). Historically, the extremity paresthesias have been observed to be independent of dose; there was no effect of dose and schedule in this study, although paresthesias tended to develop earlier in those receiving 500 mg of oltipraz weekly compared with those receiving 125 mg daily. All of the adverse events were transient, resolving within days of last dose. In addition, some people became tolerant. Of the seven individuals who were rechallenged with oltipraz after initial discontinuation because of paresthesias, none reported exacerbation of symptoms, and all but one reported diminution or no subsequent effects. Because of the frequency with which this adverse event was reported and the degree of discomfort engendered, follow-up studies on the etiology of this effect and development of approaches for symptomatic relief should be considered before oltipraz undergoes expanded evaluation in large Phase II or Phase III chemoprevention studies. As in the previous studies, abnormal laboratory values (hematology, chemistry profiles) were not attributed to the drug.

One argument against the generalization of these results to the underlying population was the excessive exclusion during screening. Because the participants were community volunteers, the proportion excluded due to health problems noted at screening was unexpected. One potential explanation is that the standards for defining healthy normal limits may not be applicable to this population. The individuals in Daxin were not ill, but due to genetic predisposition and nutrition may have other normal limits. For example, the overall distributions of platelets (mean ± SD = 114, 419 ± 37, 502 per mm³) and hemoglobin by gender (mean ± SD = 14.5 ± 1.4 mg/dl and 12.5 ± 1.4 mg/dl for males and females, respectively) were lower than the standard distributions for United States adults. A standard distribution for the Daxin population was not available, but clinicians in the People’s Republic of China considered the values to be within normal limits for the population. The method of field collection of the samples and subsequent 2-h delay to assay, occurring in hot weather, also may have resulted in falsely lower platelet counts. A rise in platelet measurements (mean ± SD = 169, 216 ± 48, 929 per mm³) observed at week 17, when the collection protocol changed and the weather became cooler, provided reassurance for the decision to allow HBsAg-negative individuals with platelet values as low as 75,000/mm³ to enroll. The lack of trends in adverse events and attrition according to baseline physiological measures gives some credence to this explanation. Other potential reasons for the disproportionate representation of those with medical problems in the screened population include an overenumeration by the field doctors and an increased self-inducement to participate. Given the large ineligible proportion, either relaxed criteria or a larger screening population will have to be used when planning other Phase II or III trials of similar magnitude in this population. The observed exclusion, however, did not affect the representation of the study group to the underlying population, except for the percentage who were HBsAg positive. This underrepresentation, due to more abnormal liver function test values, was not unexpected since this population was chosen because of its increased risk of liver disease.

Given the multiplicative interaction between aflatoxin biomarkers and infection with HBV in the risk of HCC observed previously (3, 4), it would be highly beneficial to target these individuals when implementing such a chemoprevention strategy in the population. An intolerance to the drug would prohibit participation. Of the four HBsAg-positive individuals on daily active drug, one reported mild nausea and one had elevated liver function tests; none of the four receiving 500 mg of oltipraz weekly experienced any adverse events. Although more HBsAg-positive individuals need to be studied for conclusive results, the data from this study argue against their exclusion from future trials.

The good compliance with the study protocol demonstrates that, despite the intense follow-up schedule typically required in clinical trials with biomarker end points, such studies may be successfully conducted in similar populations. A more complete understanding of the chemopreventive utility of oltipraz will await an assessment of its efficacy in modulating the aflatoxin biomarkers.

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