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Oltipraz Chemoprevention Trial in Qidong, Jiangsu Province, People's Republic of China

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Abstract Oltipraz has been used clinically in many regions of the world as an antischistosomal agent and is an effective inhibitor of aflatoxin hepatocarcinogenesis in rats. This chemopreventive action of oltipraz results primarily from an altered balance in aflatoxin metabolic activation and detoxication. In 1995, a randomized, placebo-controlled, double-blind intervention was conducted in residents of Qidong, People's Republic of China, who are at high risk for exposure to aflatoxin and development of hepatocellular carcinoma. The major study objectives were to define a dose and schedule for oltipraz that would reduce levels of aflatoxin biomarkers in biofluids of the participants, and to further characterize dose-limiting side effects. Two hundred thirty-four healthy eligible individuals, including those infected with HBV, were randomized to receive either 125 mg oltipraz daily, 500 mg oltipraz weekly, or placebo. Blood and urine specimens were collected to monitor potential toxicities and evaluate biomarkers over the 8-week intervention and subsequent 8-week follow-up periods. Overall, compliance in the intervention was excellent; approximately 85% of the participants completed the study. Objective evaluation of adverse events was greatly facilitated by inclusion of a placebo arm in the study design. A syndrome involving numbness, tingling, and pain in the fingertips was the only event that occurred more frequently among the active groups (18 and 14% of the daily 125 mg and weekly 500 mg arms, respectively) compared to placebo (3%). These symptoms were reversible and could be relieved with non-steroidal antiinflammatory agents. A more complete understanding of the chemopreventive utility of oltipraz awaits completion of an assessment of the efficacy of oltipraz in modulating levels of aflatoxin biomarkers. J. Cell. Biochem. Suppls. 28/29:166–173.

Key words: aflatoxin B_1; aflatoxin albumin adducts; biomarkers; enzyme induction; glutathione *S*-transferases; hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most common cancers in China and results in an estimated 200,000 deaths annually. HCC is the leading cause of cancer death in Qidong County in eastern Jiangsu Province, People's Republic of China (PRC), and accounts for up to 10% of all adult deaths in some of the rural townships [1,2]. Case-control studies in this region indicate that chronic infection with Hepatitis B virus (HBV) is an important risk factor. However, while 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

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to aflatoxins in the diet and algal toxins in the drinking water also contribute to the extraordinarily high risk of HCC in Qidong City [1,2]. 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. An ongoing nested case-control study in nearby Shanghai has demonstrated a multiplicative interaction between HBV and aflatoxins in risk of HCC [3,4]. A recent longitudinal survey of 120 residents of Daxin Township, Qidong City, indicated that greater than 95% of the participants tested positive for serum aflatoxin albumin adducts throughout a 3-month period [5]. Molecular studies also suggest 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 [6]. 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 aflatoxin B_1 leads to preferential G to T transversion mutations of the third base in codon 249 [7].

Strategies for the primary prevention of HCC in Qidong City include HBV vaccination programs, improved water quality and crop control, and diminished consumption of corn [1,2]. However, to break the cycle that begins with HBV infection at birth, universal vaccination must be carried out for several generations. As a consequence, the desired effect of reducing HCC may take some time to emerge. Cost also greatly restricts the use of HBV vaccines. The extent of aflatoxin contamination in foods is a function of the ecology of molds and is neither completely preventable, nor is prevention economically feasible, in much of the world. Thus, in practice, additional strategies need to be developed to impart immediate, significant worldwide impact upon mortality rates of HCC. Secondary prevention programs, such as chemoprevention, may be useful in this context. Experimentally, aflatoxin-induced hepatocarcinogenesis can be inhibited by over a score of different chemopreventive agents [8,9]. One of the most potent and effective agents in these animal models is the antischistosomal drug, oltipraz (4-methyl-5-(N-2-pyrazinyl)-1,2-dithiole-3-thione).

MECHANISM-BASED SELECTION OF OLTIPRAZ

Efficacy and Mechanisms in Animal Models

Oltipraz was extensively evaluated as a treatment for schistosomiasis in the early 1980s. Single doses of oltipraz have achieved cure rates of greater than 90%. While studying the schistosomicidal activity of oltipraz, Bueding and colleagues, 1986 [10] noted that administration of this drug to mice resulted in marked elevations of the activities of phase 2 enzymes in hepatic and extrahepatic tissues. These findings led Bueding to predict that oltipraz would be an excellent candidate compound for cancer chemoprevention studies. Over the past decade, oltipraz has proven to be an effective anticarcinogen in breast, colon, pancreas, lung, forestomach, skin, bladder, and liver tumor models [11]. Roebuck et al., 1991 [12] reported that dietary administration of oltipraz (750 ppm) afforded complete protection against aflatoxin-induced hepatocarcinogenesis when administered before and during the period of carcinogen exposure. Subsequently, this group has shown that an intervention with oltipraz that is delayed and transient with respect to aflatoxin administration can nonetheless reduce the incidence of HCC in rats [13]. In this instance, aflatoxin was administered daily for 5 weeks while oltipraz (500 ppm) was included in the diet for weeks 2 and 3 only. The incidence of HCC was reduced from 83 to 63% by the oltipraz intervention. There was no indication in either of these bioassays that oltipraz shifted target organ specificity of aflatoxin from the liver to other tissues.

The protective actions of oltipraz are thought to result primarily from an altered balance between the activation and detoxication of aflatoxin B_1 (AFB_1) in the hepatocyte. As outlined in Figure 1, anticarcinogenic concentrations of oltipraz in the diet can markedly induce activities of glutathione *S*-transferases in rat tissues to facilitate conjugation of glutathione to aflatoxin-8,9-oxide, thereby enhancing its elimination and coordinately diminishing DNA adduct formation [14]. Feeding oltipraz to rats increases the specific activity of hepatic glutathione *S*-transferases, leading to commensurate elevations in the initial rate of biliary elimination of the aflatoxin-glutathione conjugate. Molecular studies indicated that initial increases in hepatic glutathione *S*-transferase mRNA and

protein levels in response to oltipraz were mediated through transcriptional activation of transferase genes [15,16]. Induction of glutathione *S*-transferases by oltipraz in primary cultures of human hepatocytes has been observed [17]. Oltipraz can also influence cytochrome P450 activities. Western blotting indicates small increases in several forms of P450 following oltipraz treatment in vivo [18]. Perhaps more notable, direct addition of oltipraz to rat microsomes inhibits AFB_1 oxidation [19]. Inhibition of CYP1A2 and 3A4 by oltipraz results in the reduction of aflatoxin metabolism to the 8,9-oxide and the hydroxylated metabolite aflatoxin M_1 in primary cultures of rat and human hepatocytes [20]. Urinary excretion of aflatoxin M_1 also drops dramatically immediately following oltipraz administration to aflatoxin-treated rats [21]. Thus, both inhibition of cytochrome P450s and induction of electrophile detoxication enzymes are likely to contribute to chemoprevention by oltipraz, although kinetic arguments suggest the latter could be more important than the former.

A practical implication of a mechanism of action involving enzyme induction arises from the long biological half-life of the enzyme inductive response. Although the half-life of oltipraz in rodents and man is <6 hours, the inductive effects on some phase 2 enzymes persists for over 1 week. Thus, intermittent dosing schedules may offer advantages (fewer side effects, greater compliance) while maintaining efficacy (enhanced carcinogen detoxication). As a result, the effect of dose scheduling on inhibiting aflatoxin-induced tumorigenesis has been evaluated. Rats were treated with AFB_1 daily for 4 weeks and oltipraz either daily, once-weekly, twice-weekly, or not at all throughout this period. All three intervention schedules with oltipraz engendered >95% reductions in hepatic tumor burden [22].

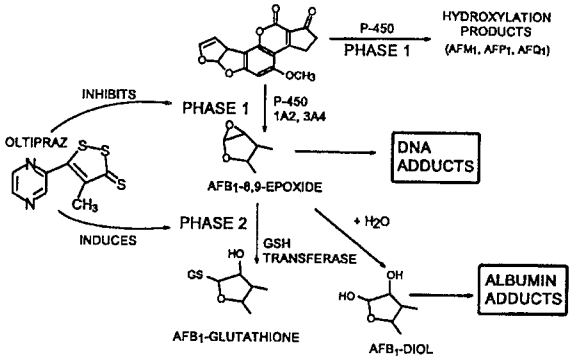

Fig. 1. Effect of oltipraz on the metabolism of aflatoxin B_1 and biomarkers of the biologically effective dose: Aflatoxin-DNA adducts and aflatoxin-albumin adducts.

Pharmacodynamic Action in Humans

Several Phase I chemoprevention studies have been recently conducted with oltipraz to define potential dose-limiting side-effects and to characterize the pharmacology of the drug. Initial studies on the pharmacodynamic action of oltipraz examined the elevation over baseline of glutathione levels and glutathione *S*-transferase activity in lymphocytes of participants receiving either 100 or 125 mg oltipraz [23]. Enzyme induction was seen in both dose groups. O'Dwyer et al, 1996 [24] have subsequently examined the effects of single oral doses of oltipraz on the expression of phase 2 enzyme genes in both lymphocytes and colonic mucosa. mRNA content for NAD(P)H:quinone reductase and γ-glutamylcysteine synthetase increased 4- and 6-fold, respectively, in colonic mucosa 2–4 days after treatment with 250 mg/m^2 oltipraz. Strong correlations were seen between the mRNA increases observed in colonic mucosa and peripheral lymphocytes. Thus, at least some of the protective mechanisms affected by oltipraz in experimental models appear to be recapitulated in humans.

OLTIPRAZ CHEMOPREVENTION TRIAL: GENERAL DESIGN AND STRUCTURE

The striking activity of oltipraz in experimental models coupled with its extensive preclinical and clinical development has provided the opportunity to use a mechanism-based approach for the design and conduct of a chemopreventive intervention in individuals at high risk for exposure to aflatoxins and development of HCC. The Oltipraz Chemoprevention Trial was a randomized, placebo-controlled, double-blind study with the primary objective of defining a dose and schedule for oltipraz that would reduce levels of aflatoxin adduct biomarkers in urine and/or serum compared to placebo. A second objective was to confirm the maximum safe dose of oltipraz following chronic exposure. A synopsis of the design of the clinical trial is shown in Figure 2. The design of the trial was to randomize 240 adults in good general health without any history of major chronic illnesses and with detectable serum aflatoxin adduct levels at baseline, into three intervention arms:

placebo; 125 mg oltipraz administered daily; and 500 mg oltipraz administered weekly. To preserve double-masking, all participants received identical capsules, containing either oltipraz or excipient only as appropriate, on each day of the intervention. The trial included men and women and did not exclude Hepatitis B viral surface antigen (HBsAg) positive individuals with evidence of normal liver function. Full methodological details of this chemoprevention trial have been recently described [25].

The doses and schedules of oltipraz used in the two intervention arms were chosen on the basis of practical and mechanistic considerations. The daily dose of 125 mg oltipraz (Arm B) reflected the maximum safely tolerated dose of oltipraz from a recent Phase I chemoprevention study of 6 months' duration [23,26]. Results of pharmacokinetic studies indicated that peak plasma concentrations of approximately 1–2 μM oltipraz occurred at this dose, whereas administration of 500 mg produced peak plasma concentrations of 20 μM [23]. Studies in rodent and human cells in culture indicated that this latter concentration is sufficient to double the specific activity of a number of carcinogen detoxication enzymes [16,27]. Considerations of the pharmacokinetics (e.g., short plasma half-life) and pharmacodynamics (e.g., prolonged enzyme induction) of oltipraz described *supra* led to the selection of a weekly dose of 500 mg oltipraz for Arm C.

Recruitment, Screening, and Randomization

Study participants were recruited from Daxin Township, Qidong County, Jiangsu Province, People's Republic of China. Daxin is a rural farming community of approximately 40,000 residents. The study enlisted the assistance of the village doctors who identified potentially eligible residents and asked for volunteers to be screened for the trial. One thousand and six individuals were screened at the Daxin Medical Clinic over a 1-week period. A signed informed consent was obtained from all participants in accordance with institutional and federal guidelines in the PRC and United States. A medical history, physical examination, liver ultrasound, EKG, and routine hematological, hepatic, and renal function tests were used to screen the individuals.

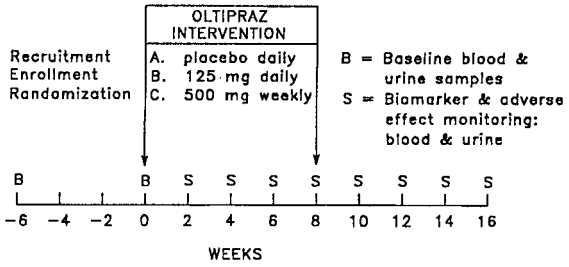

Fig. 2. Timeline for the Oltipraz Chemoprevention Trial conducted in Qidong, Jiangsu Province, People's Republic of China in May to November 1995. Reproduced from Kensler and Groopman [29] with permission from the publisher.

Individuals were excluded based on an abnormal physical examination, history of a chronic disease, α-fetoprotein positivity, abnormal liver scan or EKG, abnormal urinalysis, low blood counts, abnormal blood chemistry values (urea nitrogen, creatinine, total bilirubin, total protein, albumin, abnormal γ-GT, ALT, AST, alkaline phosphatase, and triglycerides), and outlying aflatoxin-albumin adducts ($<$1.25 or $>$10.0 pmol aflatoxin bound/mg albumin). Women who were pregnant (reported or positive β-HCG) or who were lactating also were excluded. Of the 1,006 screened individuals, 628 were excluded by at least one criterion from the initial physical examination or clinical laboratory analyses; primary reasons included liver abnormalities, renal abnormalities, abnormal hematology, and/or cardiovascular problems. An additional 18 people were excluded based upon outlying levels of aflatoxin albumin adducts. Among the 344 eligible people, 240 agreed to participate. The other 98 people mainly were unable to commit to being in the area for the duration of the trial. Eligible individuals provided informed written consent for continued participation and were assigned by the data center to one of three intervention arms using a fixed randomization scheme.

Two hundred thirty-four participants actually returned to the Daxin Medical Clinic for enrollment. All study participants remained eligible as determined on-site from another physical examination and urinalysis and received their first dose of study drug.

Follow-Up and Symptom Monitoring

Urine and blood samples, collected biweekly throughout the 8-week intervention, and a subsequent 8-week post-intervention follow-up (Fig. 2), provided the basis for monitoring toxicities and measuring aflatoxin biomarkers. Portions of each sample were shipped frozen by air-freight to Baltimore for blood chemistry analyses, as specified *supra*. All analyses were com-

pleted within 96 hours of blood collection with results forwarded to the Qidong Liver Cancer Institute. Laboratory measurements and self-reported symptoms were used to assess potential toxicities. The criteria of Tangrea et al., 1991 [28] were used to record the participant's subjective assessment of severity. In addition to the daily visits by the village doctors for dispersal of study drugs, a symptom report was completed weekly by interviewing each participant, to standardize the data collection and capture minor complaints that otherwise may have been missed. Physical examinations were performed at weeks 4, 8, and 16. The study drug was discontinued if symptoms or laboratory evidence of toxicities of grade 2 or 3 were encountered. Participants were followed until symptoms resolved. 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 use of the study medication. All data collection forms used in the study were bilingual.

OLTIPRAZ CHEMOPREVENTION TRIAL: OUTCOMES

Compliance/Adverse Events

Adherence to study protocol was relatively good (Fig. 3) and was facilitated through daily interaction of the participants with the village doctors. A total of 195 participants (83.3%) were administered drug through week 8 of the intervention. Of these, 132 participants took their drugs for the entire period without interruptions, distributed as 71, 47, and 50% of the placebo, 125 mg and 500 mg arms, respectively, while 192 (93, 74, and 80% of each arm) took their drugs for at least 44 days (80% of the intervention period). The attrition by intervention group was 8 in the placebo arm, 20 in the 125 mg arm, and 16 in the 500 mg arm. Twenty-eight (63.6%) of the 39 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, and 500 mg arms, respectively. The two primary reasons given for withdrawal from the other 11 participants were that they were "tired of participation" and that the participants "moved from Qidong." Only 5 other people withdrew from the study post-intervention.

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. The majority of adverse clinical events occurred shortly after initiating treatment; 73.8% of individuals on active drug with events developed their symptoms in the first week.

The distribution of symptoms by type and grade was similar to those reported in earlier studies of oltipraz toxicities [11,26] and all were resolved prior to 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 class (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 after starting the intervention. This syndrome was the only adverse event significantly more reported in the 125 mg (18.4%) and 500 mg (14.1%) oltipraz arms compared to the placebo group (2.5%) ($P = 0.002$, Fisher's exact test for 3×2 contingency table). 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, whereas it was more frequently reported among the women taking the daily dose of oltipraz. 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 >24.2, 21.8 to 24.2 and <21.8, respectively. There were no consistent trends in reporting symptoms by type of oltipraz dosing.

Biomarker Modulation

The availability of well-characterized intermediate markers reflecting the modulation of the biologically effective dose of environmental carcinogens allows the design and conduct of efficient clinical prevention trials. Thus, the

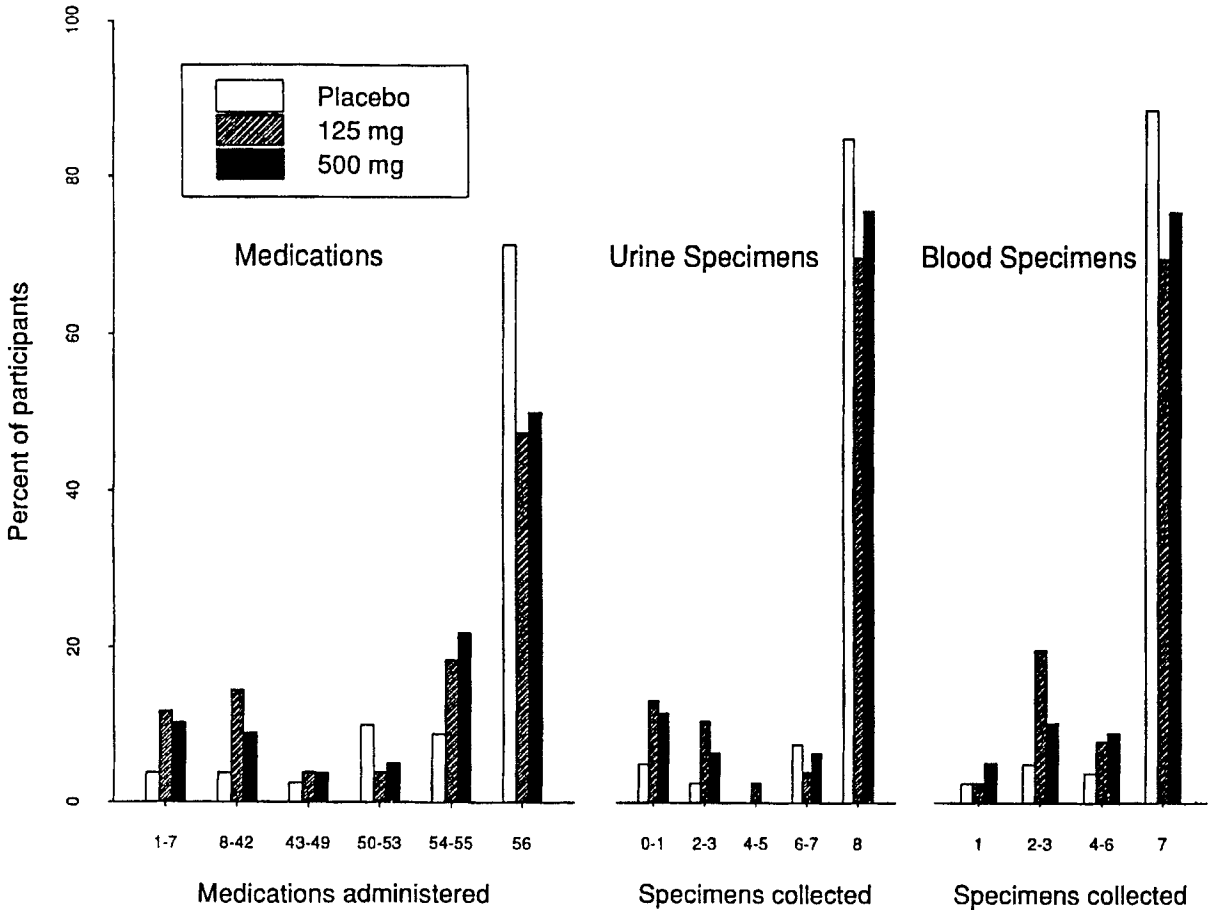

Fig. 3. Histograms describing the adherence to study protocol by intervention arm in the Oltipraz Chemoprevention Trial. Placebo = placebo intervention; 125 mg = 125 mg oltipraz administered daily; 500 mg = 500 mg oltipraz administered weekly. **Left:** Distribution of drug use by intervention arm; total possible use is 56 days. **Middle** and **Right:** Distribution of donated blood and urine specimens, respectively, over the 8-week intervention and subsequent 8-week follow-up period. The follow-up protocol consisted of 7 blood and 9 urine collections.

primary objective of the Oltipraz Chemoprevention Trial was to use several markers of the biologically effective dose of AFB_1 as modifiable endpoints to define active dose(s) and schedule(s) of oltipraz. The presence of these markers, aflatoxin-albumin adducts in serum and aflatoxin-N^1-guanine adducts in urine, reflect both exposure of people to dietary aflatoxins and increased risk for HCC. Sensitive, specific techniques amenable to large numbers of samples have been developed for these aflatoxin DNA and protein adducts. As a result, these biomarkers have undergone extensive validation in ecological and prospective epidemiological studies in the PRC and elsewhere [reviewed in 29]. Levels of these biomarkers can be readily lowered in aflatoxin-exposed animals undergoing oltipraz interventions, although the extent of their diminution often underestimates the ultimate degree of tumor reduction [12,29]. Analyses of additional urinary metabolites of aflatoxin, notably aflatoxin M_1 and aflatoxin mercapturic acid, may provide insight into the actions of oltipraz to inhibit cytochrome P450s or induce glutathione *S*-transferases, respectively. A notable advantage in the assessment of these endpoints is a study design featuring repeated samples that allows for individuals to serve as their own controls. Thus, the power of the study to detect biomarker modulations is amplified considerably beyond that afforded by simple, cross-sectional, intergroup comparisons [30].

In addition to monitoring for potential changes in aflatoxin-specific biomarkers, genotoxicity assays are being conducted on urine samples collected from each study participant during weeks 4 and 6 of the intervention. These

assays measure the internal dose of mutagenic and DNA-damaging agents excreted into this biological fluid [31]. While excreted aflatoxin metabolites are not typically genotoxic, tobacco smoking is a major source of urinary genotoxins. Smokers were prevalent in the study cohort, particularly amongst the males, thus providing the opportunity to seek additional insights into the possible spectrum of chemopreventive efficacy of oltipraz. A full evaluation of the outcome of the Oltipraz Chemoprevention Trial awaits the completion of these biomarker analyses.

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