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Mechanisms of Chemoprotection by Oltipraz

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Abstract 1,2-Dithiole-3-thiones are five-membered cyclic sulfur-containing compounds with antioxidant, chemotherapeutic, radioprotective and cancer chemoprotective properties. One substituted dithiolethione, oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione], originally developed as an antischistosomal agent, has recently been observed to protect against chemically induced carcinogenesis in lung, trachea, forestomach, colon, breast, skin, liver and urinary bladder in rodents. The induction of electrophilic detoxication enzymes, which result in diminished carcinogen-DNA adduct formation and reduced cytotoxicity, appears to be an important component of the anticarcinogenic action of oltipraz and other dithiolethiones. Phase I trials of oltipraz are presently underway in the United States. Subsequent trials might be most appropriately targeted towards individuals at high risk for occupational or environmental exposures to genotoxic carcinogens.

Key words: 1,2-dithiole-3-thiones, antischistosomiasis, carcinogen detoxication, DNA adducts, enzyme induction, glutathione *S*-transferases, oltipraz

Several substituted 1,2-dithiole-3-thiones exhibit chemotherapeutic, radioprotective and chemoprotective properties [1–4]. One of these agents, oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione] (Fig. 1), has shown significant antischistosomal activity in experimental animals and in humans. Cure rates of up to 90% have been achieved in field trials with single doses of oltipraz. During studies on the mechanisms of antischistosomal activity of oltipraz, Bueding *et al.* [5,6] noted that administration of the drug to mice infected with *Schistosoma mansoni* caused a reduction in the glutathione stores of the worms, but increased levels of glutathione in many tissues of the host. Subsequent studies in rodents have demonstrated that oltipraz and other related 1,2-dithiole-3-thiones were potent inducers of enzymes involved in the maintenance of reduced glutathione pools as well as enzymes important to electrophile detoxication, notably, elevated NAD(P)H:quinone reductase, epoxide hydratase, glutathione *S*-transferase (GST) and UDP-glucuronosyl transferase (UGT). The elevation of electrophile detoxication enzymes as exemplified by the antioxidants BHA, BHT and ethoxyquin has been recognized as characteristic of the action of many chemoprotective agents [7]. Similarly, oltipraz has been shown to be an effective chemoprotective agent in up to a dozen different models of experimental carcinogenesis. This broad range of anticarcinogenic activity coupled with its apparently low mammalian toxicity has prompted the continued development of oltipraz as a potential human chemoprotective agent. Oltipraz is currently undergoing Phase I clinical trials in the United States to determine its pharmacokinetic properties and dose-limiting side-effects during chronic administration [8]. This review focuses on the potent chemoprotective mechanisms of oltipraz. Recommendations are based on pharmacokinetic and mechanistic

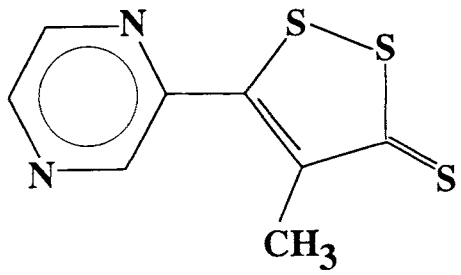

Fig. 1. Structure of oltipraz [5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione].

considerations in evaluating oltipraz in clinical chemoprotection trials in populations at high risk for exposure to genotoxic carcinogens.

CHEMOPROTECTION IN EXPERIMENTAL MODELS

The biochemical manifestations of oltipraz in schistosome-infected mice prompted Bueding [5] to predict it might have cancer chemoprotective properties. Initial confirmation that 1,2-dithiole-3-thiones may exert chemoprotective effects *in vivo* came from demonstrating that oltipraz protected mice against the hepatotoxicity of carbon tetrachloride and acetaminophen [9]. In subsequent studies, oltipraz was effective against the acute hepatotoxicities of allyl alcohol and acetaminophen in hamsters [10,11] and aflatoxin B_1 (AFB_1) in rats [12]. Toxin-induced elevations in liver function tests were blunted in all cases. Pretreatment with oltipraz also substantially reduced the mortality produced by either single or chronic exposure to AFB_1 [12; Maxuitenko and Roebuck, unpublished observations].

To test the cancer chemoprotective activity of oltipraz directly, Wattenberg and Bueding [13] examined the ability of oltipraz to inhibit carcinogen-induced neoplasia in mice. Oltipraz was administered either 24 or 48 hours before treatment with each of three chemically diverse carcinogens: diethylnitrosamine, uracil mustard, and benzo[*a*]pyrene. The sequence of oltipraz and carcinogen administration was repeated weekly for 4 to 5 weeks. Oltipraz reduced the number of benzo[*a*]pyrene-induced pulmonary adenomas and tumors of the forestomach by nearly 70% each. Pulmonary adenoma formation induced by uracil mustard or diethylnitrosamine was also significantly reduced by oltipraz pretreatment, but to a lesser extent. Subsequent studies have shown oltipraz to have chemoprotective activity against different classes of carcinogens targeting the breast [14,15], colon [16], liver [17], skin [18], trachea [14] and bladder [14]. Unfortunately, the full experimental details of only a few of these studies have been published, rendering comparison of the potency and efficacy of oltipraz to other chemoprotective agents affecting the same target sites difficult. Nonetheless, the potency of oltipraz is demonstrated by observing that dietary concentrations of 200 and 400 ppm oltipraz significantly reduced tumor incidence and multiplicity in azoxymethane-induced intestinal carcinogenesis in F344 rats [16], while 750 ppm dietary oltipraz afforded complete protection against AFB_1-induced hepatocarcinogenesis [17]. Moreover, dietary concentrations as low as 100 ppm resulted in >90% reductions in the hepatic burden of the presumptive preneoplastic lesions in the aflatoxin model [19]. To date, one negative study with oltipraz has been reported. Pepin *et al.* [20] observed no effect on pulmonary tumorigenesis induced by the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after feeding mice 250 ppm oltipraz. Attenuation of the effects of tobacco-specific carcinogens in target organs such as the pancreas and the bladder remains to be established.

The role of oltipraz in combination with other presumptive chemoprotective agents is also under investigation. Mice fed either 40 or 80% of the maximum tolerated dose of oltipraz showed no effect in the prevention of urinary bladder carcinogenesis induced by *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine. However, when oltipraz was combined with α-difluoromethylornithine, significant dose-dependent inhibition was observed [21]. Oltipraz in combination with either the retinoid *N*-(4-hydroxyphenyl)retinamide or β-carotene was also very effective against diethylnitrosamine-induced respiratory carcinogenesis [22]. Thus, the use of oltipraz in combination with agents exhibiting different mechanisms of action appears promising.

MECHANISMS OF CHEMOPROTECTION

Although oltipraz exerts chemoprotective activity in a variety of animal models, very few

studies have addressed possible anticarcinogenic mechanisms in these systems. All of the chemoprotection protocols tested to date have involved concomitant exposure to both a carcinogen and oltipraz. Oltipraz may affect the metabolism and/or disposition of carcinogens (Fig. 2). Possible mechanisms to explain the observed protective effects of oltipraz may include (1) inhibition of Phase I enzymes to retard metabolic activation; (2) induction of Phase I enzymes (*i.e.*, cytochrome P-450s) to enhance carcinogen detoxication; (3) induction of Phase II xenobiotic-metabolizing enzymes (*e.g.*, GSTs, UGTs) to enhance carcinogen detoxication and elimination; (4) nucleophilic trapping of reactive intermediates; and (5) enhancement of DNA repair processes. Post-initiation effects (6) of oltipraz may also occur but have not been extensively examined in anticarcinogenesis bioassays.

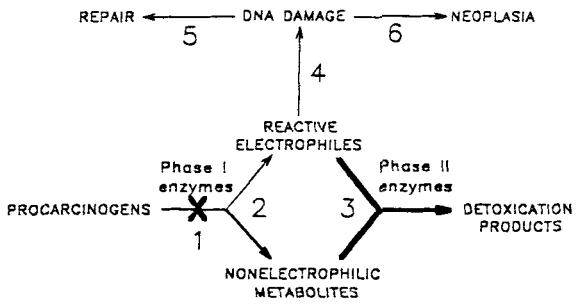

Fig. 2. General effects of oltipraz on carcinogen metabolism and disposition. Numbers indicate possible mechanisms of action; see text for details.

Oltipraz inhibits AFB_1-induced hepatocarcinogenesis by modifying the metabolism and disposition of the carcinogen. In particular, alterations in the balance of competing pathways of the ultimate carcinogenic agent, aflatoxin-8,9-oxide, directly modulate the availability of the epoxide for binding DNA. Anticarcinogenic concentrations of oltipraz in the diet markedly induce the activities of GSTs in rat tissues. This facilitates the conjugation of glutathione to aflatoxin-8,9-oxide, thereby enhancing its elimination and diminishing DNA adduct formation [19,23]. Feeding 750 ppm oltipraz for 1 week before exposure to AFB_1 increases the initial rate of biliary elimination of the aflatoxin-glutathione conjugate nearly three-fold [24]. These rats showed a three- to four-fold increase in the specific activity of liver GST [19]. *In vitro* studies have shown that purified rat alpha class GSTs will conjugate aflatoxin-8,9-oxide to glutathione [25]. Three-fold elevations in hepatic levels of the alpha GST subunit Ya have been measured 3 to 7 days after the inclusion of dietary oltipraz [26]. Slot blot analysis using a full-length cDNA probe for the rat GST Ya gene showed elevated steady state levels of hepatic GST Ya mRNA in response to oltipraz. Nuclear runoff experiments suggested that the initial increases in hepatic GST Ya mRNA and protein levels were modulated by transcriptional activation of the GST Ya gene in response to oltipraz [26]. Several regulatory elements controlling the expression and inducibility of the Ya subunit of rat GST have been identified [27]. A 41 base pair (bp) element in the 5′-flanking region of the rat GST Ya gene, termed the "Antioxidant Response Element," appears to mediate induction by oltipraz. The molecular details of the signaling pathway for inducing GST Ya and other Phase II enzymes by oltipraz or other 1,2-dithiole-3-thiones remain to be fully established. While the role of GSTs as determinants of the sensitivity of the liver to AFB_1-induced hepatocarcinogenesis appears reasonable, other Phase II enzymes, notably, UGTs and NAD(P)H:quinone reductase, are likely to be important in the detoxication of other carcinogens; these need to be explored in greater detail. A significant attribute of oltipraz is the response in a variety of tissues (*e.g.*, liver, lung, intestinal mucosa, kidney, bladder) to its enzyme-inductive actions.

Oltipraz is a very weak inducer of some cytochrome P-450 enzymes. Incubation of AFB_1 with microsomes prepared from oltipraz-fed rats produces a moderately increased amount of some oxidative metabolites as compared to control microsomes [19], although no increases in aflatoxin-8,9-oxide have been observed in hepatic microsomes from oltipraz treated rats [28]. Putt *et al.* [28] evaluated the effect of oltipraz on the induction of different classes of cytochrome P-450s, as determined by Western blot. There was no effect on CYP 1A1, 2B1, 2C12, 3A1/2 and 3A4 levels, and only small increases in 2C11 and 2E1 levels. Direct addition of oltipraz to untreated microsomes also affects the metabolism of AFB_1. Addition of 100 μM oltipraz to microsomal incubations results in substantial inhibition of the forma-

tion of aflatoxin-8,9-oxide [28]. The nature of this inhibition has not yet been characterized, nor is it known whether pharmacologically achievable concentrations in the liver affect cytochrome P-450 activities *in vivo*. Although oltipraz reduces the levels of aflatoxin-8,9-oxide *in vitro*, it does not appear to function as a direct nucleophilic trap. This conclusion is supported by studies showing no differences in the levels of aflatoxin-N^7-guanine as measured by HPLC when aflatoxin-8,9-oxide is added directly into a solution containing calf thymus DNA in the absence or presence of excess amounts of oltipraz. Moreover, chromatographic analyses provide no evidence for the formation of aflatoxin-oltipraz adducts [24].

Oltipraz and other electrophile detoxication enzyme inducers clearly protect against the DNA-damaging actions of procarcinogens; however, such mechanisms cannot explain anticarcinogenic effects in models where direct-acting carcinogens are used (*e.g.*, MNU) [15]. Elevation of cellular thiol levels may lead to the trapping of reactive intermediates. Moreover, it is very apparent from molecular dosimetry studies in the aflatoxin model that reduction in the target organ DNA adduct burden substantially underestimates the ultimate degree of chemoprotection against disease endpoints [19,29]. It is important to consider, therefore, additional actions by these agents. Cohen and Ellwein [30] have recently examined the importance of cell proliferation in the neoplastic process. They have noted a modest dose-dependent rate of increase in tumor prevalence at low doses of carcinogens as a consequence of DNA interactions; whereas at higher doses, a much greater rate of increase reflects the synergistic influence of increased cell proliferation. The cytotoxic actions of these carcinogens produces increased compensatory proliferation of surviving cells. The powerful protective effect of oltipraz against the cytotoxic actions of hepatotoxins suggests this agent may act indirectly to reduce cell proliferation, thereby enhancing its anticarcinogenic activity.

Still to be addressed is whether or not oltipraz exerts any post-initiation effects. Preliminary observations in our laboratories indicate that oltipraz does not affect phorbol ester-mediated proliferation and/or differentiation events in murine keratinocytes such as seen with retinoids, non-steroidal anti-inflammatory drugs, protease inhibitors, antioxidants, or other classes of antipromoters. Moreover, feeding oltipraz to rats *following* treatment with AFB_1 appears to have no effect on the hepatic burden of presumptive, preneoplastic lesions [Maxuitenko and Roebuck, unpublished observations]. The full spectrum of anticarcinogenic mechanisms of oltipraz is far from resolved.

PROSPECTS FOR HUMAN INTERVENTIONS WITH OLTIPRAZ

The potent chemoprotective activity of oltipraz, its low toxicity in animals, and an understanding of its mechanisms of action has led to interest in oltipraz as a potential chemoprotection agent in humans and permits the rational design of clinical trials. In addition to the choice of an intervention agent, a number of issues must be considered in the design of a clinical chemoprotection trial including (1) the dose to be used, (2) the stage of the carcinogenesis process to be interrupted, (3) the appropriate outcome measure, and (4) the study population. These issues are clearly interrelated and the choice of one heavily influences subsequent choices in designing a trial.

Studies in experimental models and ongoing Phase I trials should resolve a number of practical issues including dose and scheduling parameters for optimal bioavailability, the development of indices of pharmacodynamic action (*e.g.*, Phase II enzyme induction in peripheral blood cells), and identification of potential side-effects. Pharmacokinetic studies conducted during both the single, high-dose antischistosomal trials and the current chronic, low-dose Phase I trials suggest that it should be possible to achieve chemoprotective concentrations of oltipraz in humans. A major issue still to be resolved is the identification of the appropriate high-risk populations that might benefit most from oltipraz intervention. Autoradiographic studies in animals indicated that the highest concentrations of oltipraz were found in the gastrointestinal tract, liver, kidneys and bladder. Consequently, these tissues are likely to represent the most promising targets for chemoprotection by oltipraz. Cancer chemoprotection in animals has already been achieved in most of these tissues. Mechanistic studies indicate that

individuals most likely to benefit from oltipraz are those exposed to carcinogens susceptible to detoxication by Phase II enzymes. As a consequence, trials examining the efficacy of oltipraz might select participants from populations exposed to heavy concentrations of airborne pollutants, dietary aflatoxins, or chemotherapeutic alkylating agents.

Cancer of the urinary tract is a significant health problem worldwide and represents 10% of all cancers reported in the U.S. Occupational exposure to chemicals, particularly aromatic amines and nitroaromatics, has long been implicated as important etiologic factors, and workers in the dye, leather, plastics, rubber and transportation industries remain at elevated risk for bladder cancer [31]. Numerous studies have also demonstrated approximately double the risk of bladder cancer in smokers as compared to nonsmokers [32]. While the carcinogenic components of tobacco may include polycyclic aromatic hydrocarbons, *N*-nitrosamines and aromatic amines, the specific etiologic agents are unknown. Oltipraz is known to inhibit carcinogenesis induced by polycyclic aromatic hydrocarbons and *N*-nitrosamines in several organs including the bladder; anticarcinogenic effects against aromatic amines have not as yet been evaluated. Other risk factors for bladder cancer include exposure to radiation, cyclophosphamide, and infection with *Schistosoma haematobium* [31,33]. A particularly intriguing aspect of using oltipraz in populations at high risk for bladder cancer is its antischistosomal activity. In general, antischistosomal *versus* anticarcinogenic activities can be readily dissociated (*i.e.*, many anticarcinogenic 1,2-dithiole-3-thiones are devoid of antischistosomal activity); thus, these dual activities might be unique to oltipraz and may lend prophylactic advantage to its use.

Because the natural history of urinary bladder carcinogenesis involves a wide and variable latency period [33], there are multiple opportunities for intervention. Chemoprotection trials aimed at interrupting the early stages of carcinogenesis have largely been avoided; however, strategies to inhibit the genotoxic actions of occupational and tobacco-related exposures throughout the disease process might be of significant benefit. Such studies are greatly facilitated by the availability of intermediate biomarkers to rapidly assess the efficacy of protective interventions. In the case of bladder cancer, approximately two-thirds of the tumors at the time of diagnosis are initially localized and non-invasive. They are, however, a source of considerable discomfort, disability and expense, particularly since recurrence rates as high as 80% are reported and many of these tumors subsequently become invasive [34]. The presence of superficial, noninvasive tumors with a high recurrence rate make bladder cancer particularly suitable for chemoprotection studies [35]. Animal bioassay, mechanistic and pharmacokinetic studies collectively suggest that oltipraz might be an excellent candidate for chemoprotection trials in the suppression of recurrent neoplasms.

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