The chemopreventive efficacy of inhaled oltipraz particulates in the B[α]P-induced A/J mouse lung adenoma model

Sheela Sharma*, Pu Gao and Vernon E. Steele1

Cellular and Molecular Toxicology Program, Alion Life & Environmental Sciences, Research Triangle Park, NC 27709, USA and 1Division of Cancer Prevention, National Cancer Institute, NIH, Bethesda, MD 20892, USA

*To whom correspondence should be addressed at: Alion Science & Technology, PO Box 12313, Research Triangle Park, NC 27709, USA. Tel: +1 919 406 2225; Fax: +1 919 549 9058; Email: ssharma@alionscience.com

This study explored the efficacy of oltipraz, a dithiolthione to prevent lung cancer by delivering it directly to the lung as inhaled particulates to obtain maximum efficacy with no toxicity. Two exposure regimens were used to compare the efficacies of early (Regimen-A) versus late (Regimen-B) intervention in prevention of lung tumorigenesis in A/J mice. Female A/J mice were exposed to 10, 30 and 100 mg/m3 exposure concentrations of oltipraz for 1.0 h a day for 5 days per week for 4 weeks in Regimen A. During the second and third week, mice received totally 6 mg of B[α]P via gavage and after 16 weeks, they were killed for tumor counting and pathology. In Regimen B, mice were treated first with B[α]P and, after a gap of 4 weeks, exposed to oltipraz at 100 mg/m3 for 16 additional weeks. At 22 weeks, animals were killed and necropsied for tumor scoring. The spontaneous tumors were few in untreated A/J mice (0.7 tumors/lung), whereas there was an average of 16.5 tumors per lung in the B[α]P group (20-fold induction). Evaluation of lung tumor multiplicity following exposure to oltipraz showed that oltipraz inhibited the tumor development in a dose-dependent manner (10–100 mg/m3) with inhibition ranging from 37 to 53% in Regimen A and 51% in Regimen B, when compared with the B[α]P group. Analysis of the tumor incidence showed that 81.5% of the animals had 10 or more tumors in the B[α]P group, whereas, in oltipraz exposure groups, there was a significant decrease in Regimen A (24–36%) and in Regimen B (42%). The data from this study show that oltipraz is an effective agent for lung cancer prevention, when it is delivered directly to the target tissue as aerosolized particulates.

Introduction

Totally 90% of lung cancer cases are related to tobacco smoking even though other etiological agents are implicated, which include commercial and industrial chemical pollutants, minerals, organic solvents, air pollutants and radiation. Benzo(a)pyrene (B[α]P) and similar polycyclic hydrocarbons are common products of incomplete burning of fossil fuel, tobacco and other organic matter, and are found throughout the environment. The amount of B[α]P per cigarette (in mainstream smoke) has been estimated at 20–40 ng (1). B[α]P is a potent, classic polycyclic hydrocarbon carcinogen in laboratory animals. It has been used to induce lung, skin, mammary and forestomach tumors in experimental animal studies. Inhalation exposure to B[α]P has been used to produce lung tumors in Syrian golden hamsters (2).

The Strain A mouse lung tumor bioassay has been extensively validated and used to screen chemicals for carcinogenicity (3–5). Positive responses have been found for members of most classes of chemical carcinogens, including polycyclic hydrocarbons, nitrosamines, nitrosoureas, carbanmates, metal salts, silylating agents, aflatoxin, vinyl chloride, ethylene dibromide, ethylene oxide, dimethylhydrazine, benzotrichloride and acrylamide (6,7). In addition, this model has been used to evaluate the chemopreventive activity of different agents by measuring their inhibition of lung cancer (8–15). This assay allows quick and simple evaluation of a number of lung tumors, producing results within 6 months, and it is in an organ system known for its high cancer incidence in humans. In addition, the model is well characterized and the response of the Strain A/J mouse to standard carcinogens has been stable for decades.

Oltipraz, a substituted dithiolthione, is a well-known inducer of phase II enzymes including glutathione transferase, NADPH-quinone reductase and UDP-glucuronosyltransferase (16,17) and is structurally related to similar chemical groups found in cruciferous vegetables. Consumption of these vegetables has been associated with decreased human cancer risk (18). Oltipraz also has been shown to have effect on phase I enzymes involved in aflatoxin B1 metabolism by inhibiting both CYP1A2 and CYP3A4 in human hepatocytes (19) or microsomal B[α]P metabolism by inhibiting CYP1A1 (20), and at low doses in animals (21). Our own cell culture studies have shown additional chemopreventive activities of oltipraz by inhibiting promoter (TPA)-induced tyrosine kinase and carcinogen (B[α]P)-DNA binding in immortalized human bronchial epithelial cells (22). Further, oltipraz has been reported to be chemopreventive in a broad spectrum of animal models, including rat liver (aflatoxin-induced), colon and mammary gland; pancreas, forestomach, mouse skin, bladder and colon; hamster trachea and lung (23–25). Oltipraz has been tested for chemopreventive activity in the mouse lung model, giving positive results for B[α]P-induced tumors when the agent was given orally (26) and negative results for B[α]P- or nicotine-derived nitrosamino ketone-induced tumors when the agent was given in the diet (27,28). This agent has been used as a drug for treatment of schistosomiasis (29) and has low toxicity in animal and phase I human studies (30,31). In a phase II clinical trial in China, oltipraz was shown to decrease the levels aflatoxin-adducts used as a biomarker of aflatoxin exposure and subsequent development of hepatocellular carcinoma (32).

Cancer chemoprevention research has a goal of identifying those agents that have the most promising combination of low toxicity-high efficacy to inhibit one or more types of tumors. In
general, since chemopreventive agents would be taken over a long period of time, it is preferred that the agent is effective when given either orally or by another non-invasive route. However, when the objective is to prevent a tumor in the lung, a tissue with a very large surface area interfacing with the environment, it is possible that an agent could be delivered by inhalation directly to the sites of its chemopreventive action. The approach of administering a drug directly to its target tissue is expected to increase efficacy while limiting toxicity. This would ensure that the concentration of an agent at its site of action will be high and the fraction of drug reaching the systemic circulation and, thereby, producing systemic toxicity or toxicity to other tissues will be minimal. There is a great deal of interest in employing this strategy with drugs that are designed to be effective in the lungs, which are clearly unique in having an immense surface area interfacing with the environment.

For effective prevention of lung cancer, it is important to obtain information on the feasibility and efficacy of direct delivery of aerosolized chemopreventive agents to the respiratory tract. In a pilot study, we have evaluated the chemopreventive efficacy of oltipraz, against the strain A/J mouse lung adenoma induced by B[alpha]P when it was delivered directly to the respiratory tract as an aerosol. That study showed a decrease of 84.3% (P = 0.0004) in the number of tumors (gross lesions) after aerosol exposure to 105 mg/m² × 1.0 h of oltipraz following a Regimen A protocol (data not shown). The objective of the current study was to evaluate the chemopreventive efficacy of oltipraz by using a top exposure dose of 100 mg/m³ followed by two additional doses (30 and 10 mg/m³), in order to observe a dose–response.

Materials and methods

Chemicals

B[alpha]P (CAS #50-32-8, 98% pure) was purchased from Sigma and stored in amber colored bottles with teflon-lined caps at −20°C. For intragastric administration, fresh chemical was dissolved in cotton seed oil at 30°C. Oltipraz (CAS #64224-21-1, Lot # 91-343-00) manufactured by Rhone Poulenc, France was received from the ICP repository. It is a bright red powder, and, in a liquid suspension, it has a mass median aerodynamic diameter (MMAD) of 7 μm. Oltipraz was micronized by Midwest Research (Kansas City, Missouri), stored at room temperature and used. The MMAD of particle size in the micronized oltipraz was ≤1.8 μm. For inhalation studies, a dry powder aerosol was generated from oltipraz.

Animals

Seven-week-old, viral antigen- and specific pathogen-free Strain A/J female mice were purchased from the Jackson Laboratory (Bar Harbor, ME) and quarantined for up to 2 weeks in a temperature-controlled facility before use. All study animals were provided with fresh deionized, chlorinated water, and pelleted, semipurified AIN-76A diet (casein 20%, DL-methionine 0.3%, cornstarch 52%, dextrose 13%, corn oil 5%, alfacel 5%, AIN mineral mixture 3.5%, AIN vitamin mixture, 1.0%, and choline bitartrate 0.2%), purchased from ICN Biomedicals, Inc. (Aurora, OH) on an ad libitum basis (except during inhalation exposures). Before the end of a 14 day acclimation/quarantine period prior to use, all mice were tail tattooed with permanent black ink, each with a unique number. Body weights were recorded weekly during chemopreventive inhalation exposure and monthly thereafter. Prior to being placed on study, all mice were placed in nose-only holding/exposure tubes and subjected to mock exposures (air) for several days using a schedule of increasing duration of restraint. Sham exposures to conditioned air were conducted on nose-only exposure restraining tubes.

Oltipraz inhalation exposure system configuration and operation

Four Cannon (Lab Products Inc., Rockville, MD) 52-port nose-only inhalation exposure systems (Figure 1) were configured to expose different groups of Strain A/J mice to three different concentrations of oltipraz. Prior to exposure, the mice were placed in individual plastic holding tubes (~2.5 cm in diameter). One end of each tube was removed and a nosepiece insert was installed and the modified tube served as holding tubes for the aerosol exposures. Each chamber had its own supply and exhaust air feeds. A continuous flow of breathing quality air was provided to the animals that were able to breathe through the open end of the tube. The airflow to each animal was set at ~250 ml/min.

Three independent Wright Dust Feeders (BGI Inc., Waltham, MA) were used to generate the three aerosol exposure concentrations (10, 30 and 100 mg/m³). The breathing air in the exposure system supplied to the animals flowed through the Wright Dust Feeder. The gearing on the Wright Dust Feeder and the packing pressure for the oltipraz slug were empirically determined and were based on the physical characteristics of oltipraz and the desired exposure concentration. The air for each dust feeder was supplied through an independent pressure regulator and needle valve connected to a central manifold. Compressed air was supplied to the manifold through a series of activated carbon scrubbers, HEPA filters and a humidifier. Independent mass flow meters (Sierra Instruments, Inc., Monterey, CA) provided accurate feedback on the total airflow through each system. The discharge port on each Wright Dust Feeder was connected directly to the upstream supply of the nose-only system.

Effluent from each nose-only system passed through a control valve to a common exhaust duct. The exhaust duct was maintained under negative pressure using a small centrifugal blower and a bleed valve. By adjusting the supply and exhaust air manifold pressures, and the supply and exhaust air flow control valves, the total air flow through each system and the static pressure in the breathing zone of the animals were regulated. The static pressure was maintained at ~0.2” H2O. A magnetic pressure/vacuum gauge was used as a continuous display monitor. Particulate material in the effluent air stream was removed by HEPA filtration. The differential pressure across the HEPA filter was monitored and served as an indicator of filter condition. Prior to the first day of exposure, each of the three Wright Dust Feeders was configured to deliver oltipraz as a dry powder aerosol. The micronized oltipraz was aerosolized and delivered to each nose-only exposure system (NOES) without further fractionation.

Monitoring of oltipraz concentration

Mass concentration was determined gravimetrically from samples collected during each animal exposure. Gravimetric samples of the oltipraz aerosol were collected, using Flow Air Samplers Series 110 Constant Flow Samplers on a 47 mm membrane filters (Nuclepore, Pleasanton, CA) from a single exposure port at a flow rate of ~250 ml/min. Filters were weighed immediately after collection on a Cahn Model C-32 ultramicrobalance (Cerritos, CA). Three samples were taken during the 1 h exposure period. The final target concentrations and inhaled doses in each exposure were calculated using the mean estimated doses of 4 and 16 weeks.

Chemoprevention study with oltipraz

Two different exposure regimens were used in this study to compare the efficacy of early (Regimen-A) versus late (Regimen-B) intervention in preventing lung tumorgenesis in A/J mice. After 1 week of quarantine, animals were acclimatized in the second week with increasing time of animal loading and holding in the nose-only exposure restraining tubes. The animals were then weighed and randomly assigned to seven treatment groups by weight. Twenty-four animals per group were used in the B[alpha]P control and oltipraz treatment groups and 12 animals per group were used in the room control, air control and vehicle control groups. In Regimen A, mice were exposed to three inhalation doses (10, 30 and 100 mg/m³) for 1.0 h per day with appropriate controls (air control ± B[alpha]P and room control ± B[alpha]P) on a 5 day per week basis for a 4 week oltipraz exposure (Table 1 and Figure 2).

Oltipraz groups were exposed to the chemical 1 week before B[alpha]P administration and exposure was continued for another 3 weeks. A room control group (B[alpha]P + Room) was used as a control for the B[alpha]P + Air group to determine whether the stress from inhalation exposure conditions affects tumor incidence. After 1 week of oltipraz exposure, mice received one dose (2 mg per mouse) of B[alpha]P in 0.2 ml cotton seed oil or 0.2 ml cotton seed oil (vehicle control) alone by intragastric inhalation within 1 h following the inhalation exposure and the exposures were continued. In the third week, the B[alpha]P treatment was repeated twice at the same dose and the exposures were continued until the end of the fourth week. The mice were held without further exposures for another 16 weeks for tumor development after which they were killed and necropsied for evaluation of lung tumors. In Regimen B mice were treated with B[alpha]P twice in week 1, and once in week 2 using the same dose that was used in Regimen A. The animals were shielded for 4 weeks and then exposed to oltipraz (1 h per day, 5 days per week), at a single concentration of 100 mg/m³ starting at week 7 for 16 additional weeks. During each 1 h period of exposure, test atmosphere samples were collected from unused exposure ports using a constant air sampling rate and the exposure
concentration was then determined gravimetrically from the mass of oltipraz collected and the volume of test atmosphere sampled.

Determination of oltipraz toxicity
The toxicity of oltipraz was determined by animal body weight change. Animals were weighed once a week during the four (Regimen A) or sixteen (Regimen B) weeks of oltipraz exposure and on a monthly basis until the termination of the study. At this time, detailed clinical observations for signs of toxicity were performed on each study animal. The animals were observed once a day for morbidity and mortality.

Pathology procedures
Animals were necropsied either at 20 (Regimen A) or 22 (Regimen B) weeks following the first B[a]P exposure. The lungs were removed en bloc from each animal, inflated and fixed in Tellyesniczky’s fixative (70% ethyl alcohol, formaldehyde, glacial acetic acid, 20:2:1); the trachea was closed with a ligature below the larynx following inflation. Gross lesions consistent with primary lung adenomas were scored in a blinded fashion by three independent technicians and recorded. In addition, lungs from the two groups exposed to oltipraz (i.e., + and − carcinogen), the group exposed to carcinogen and air only and the group exposed to air only and no carcinogen were selected for histological processing, characterization of the lung lesions and scoring of specific lesion types (i.e. hyperplasia, adenoma).

Hyperplasias were identified as focal lesions in which the alveoli were lined by a single layer of cuboidal cells containing mildly pleomorphic nuclei. Adenomas were distinguished as discrete masses generally 1 mm or more in diameter with the normal alveolar structure replaced by numerous densely packed alveolar and papillary structures composed of a single layer of cuboidal cells (33).

Statistical analysis
Numerical study data from the oltipraz chemoprevention bioassay were analyzed statistically by ANOVA one-way Student’s t-test for tumor incidence and body weight changes, Cochran–Armitage test for tumor multiplicity (34,35) using a P < 0.05 level of significance. Data files generated from spreadsheets were used for statistical analysis using an SAS program.

Results
Target concentration
During the oltipraz exposure, the concentration of oltipraz (as mg/m³) was determined gravimetrically and the data showed that the measured average oltipraz concentration for the target aerosol concentrations; i.e. 10, 30 and 100 mg/m³ A and B was 8.43 ± 1.35, 33.5 ± 4.9 and 101.34 ± 32.22 mg/m³, respectively (Table II). The exposure systems displayed good temporal concentration stability at all exposure concentrations, during the 4 week or 16 week period of exposure. These results are typical for the aerosol generation procedure employed in these exposures, using a Wright Dust Feed Generator, or for a particulate aerosol involving a chemical like oltipraz, and its physical characteristics.

Toxicity of oltipraz
For the chemoprevention studies, 100 mg/m³ was selected as the highest exposure concentration based on the data from a
In order to determine the effect of oltipraz exposure on tumor multiplicity, the average of three separate tumor counts per animal was used for statistical analysis. All the animals that survived (92%) were used for the tumor count (except the animals for pathological analysis). Comparisons were performed between air control and B[a]P± oltipraz-treated groups. In addition, the air control was compared with the B[a]P only treated group for determining the effect of B[a]P on the occurrence of lung tumors. There was very low spontaneous tumor development in A/J mice (average 0.7 tumors per lung) in vehicle control (cotton seed oil) group, whereas there was an average of 16.5 tumors per lung (20-fold induction) in B[a]P-treated group (Table III).

Within each separate experiment, the room control + B[a]P and air control + B[a]P groups were compared with one another to see if they differed in the number of tumors produced using an exact two-sided Wilcoxon rank sum test. The results showed that they did not differ in any of the three experiments at the 5% significance level. However, oltipraz inhibited the tumor development in a dose-dependent manner with inhibition ranging from 37 to 53%, when compared with the B[a]P only treated group. Comparing the effect of 100 mg/m³ exposure, Regimen B showed similar inhibition response as Regimen A (51% versus 53%).

Effect of oltipraz exposure on B[a]P-induced tumor incidence

The tumor incidence data were based on the analysis of mice with tumors (Table IV) after they were subdivided into four categories: 0–3, 4–6, 7–10 and >10 tumors. The statistical analysis by the (exact) Cochran–Armitage test for trend was done by comparing the B[a]P± oltipraz-treated group to the oltipraz-treated groups. Analysis of the tumor counts in B[a]P only group showed that, 81.5% of the animals had 10 or more tumors, whereas, in oltipraz-exposed groups, there was a significant decrease in the number of animals with 10 or more tumors in a dose-dependent manner. Even though Regimen B was not as effective as Regimen A (3.5-fold reduction when compared with B[a]P group) in the percentage of animals with >10 tumors, there was ~2-fold reduction in Regimen B at the 100 mg/m³ dose (Table IV and Figure 3).
Table IV. Tumor incidence in different oltipraz exposure groups

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of animals with tumors of</th>
<th>Percentage of animals with &gt;10 tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–3</td>
<td>4.0–6.0</td>
</tr>
<tr>
<td>B[a]P only</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-Regimen A</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>30-Regimen A</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>100-Regimen A</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>100-Regimen B</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vehicle control</td>
<td>34</td>
<td>0</td>
</tr>
</tbody>
</table>

Regimen A (pre-initiation), Regimen B (post-initiation).

Fig. 3. Effect of oltipraz on high tumor incidence. The tumor incidence data were based on the analysis of mice with tumors after they were subdivided into four categories: 0–3, 4–6, 7–10, and >10 tumors. The statistical analysis by the (exact) Cochran–Armitage test for trend was done by comparing the B[a]P-treated group with the oltipraz-treated groups.

Pathology observations

Oltipraz treatment seems to have modified the type of lesions in every exposure concentration. For example, either no lesions were noticed or, if they were present, they consisted mostly of alveolar epithelial hyperplasia (minimal). In order to confirm whether the obvious effect in gross tumor counts in the oltipraz group is lesion related and determine whether the alveolar epithelial hyperplasia observed in certain dose groups are statistically significant, the number of lesion types in each lung (e.g., hyperplasia and adenoma) were scored from all surviving animals in B[a]P only control, B[a]P + oltipraz groups and analyzed for statistical significance. Three animals were used for pathological observation.

There was no difference between groups for hyperplasia (data not shown), whereas there was a significant reduction of adenomas in the oltipraz-treatment group. Comparing the exposure concentration of 100 mg/m³, Regimen B showed more inhibition than Regimen A (85% versus 77%). However, it is important to note that this observation is based on a single dose tested in Regimen B (Table V).

Discussion

In this inhalation study, the test doses of oltipraz were selected based on our previous pilot study, where we have tested aerosolized oltipraz against B[a]P-induced pulmonary adenoma formation in A/J mice. Female A/J mice were exposed to three inhalation doses of oltipraz (105, 210 and 420 mg/m³) or air for 1.0 h with appropriate controls (air control ± B[a]P) for 4 weeks. Mice received a single carcinogen treatment (B[a]P, 150 mg/kg body wt) via intragastric intubation, after 1 week of oltipraz exposure. After 4 weeks of oltipraz exposure, the mice were killed 28 weeks later. The tumor incidence data indicated a significant decrease (84.3%, (P-value = 0.0004) only in the lowest oltipraz exposure concentration (105 mg/m³). In addition, the effect of oltipraz exposure on tumor multiplicity showed that there was a significant change in tumor multiplicity (decrease in the number of animals with the highest number of tumors) in oltipraz (105 mg/m³)–treated animals when compared with the B[a]P alone control (P = 0.036). Therefore, in order to find out whether we can observe a dose-related response, 100 mg/m³ was selected as the top dose in the current study, followed by two lower doses (30 and 10 mg/m³). The data presented here clearly indicates that oltipraz can exhibit dose-dependent inhibition of pulmonary adenoma by acting in the pre-initiation phase and that the dose can be as low as 1.2 mg/kg animal body weight. In addition, as the data on oltipraz efficacy at the highest exposure concentration (100 mg/m³) during pre-initiation and post-initiation phase is similar (~51–53% inhibition), it is highly desirable to develop it for clinical trials on lung cancer prevention. No mortality or any sign of toxicity was observed during the chemoprevention study, and body weight gain in exposed animals was comparable with the corresponding control animals. There is substantial data available regarding the toxicity of oltipraz in animals. It showed a low oral toxicity in mice (oral LD₅₀ > 5000 mg/kg), and no toxicity was reported when rats were repeatedly dosed with 150 mg/kg (oral) during a subchronic study. A 1 year oral toxicity study in rats (36) has indicated that the no effect level (NOEL) in rats was 10 mg/kg body wt per day with a significant increase in liver weight at the two higher doses (30 and 60 mg/kg body wt per day). Similarly in dogs, the NOEL was 5 mg/kg body wt per day with substantial weight loss at the two high doses (15 and 60 mg/kg body wt per day).

The B[a]P-induced mouse lung adenoma bioassay has been used in a number of published chemoprevention studies. Oltipraz has been tested in the mouse lung model, giving positive results for B[a]P-induced tumors when the agent was given orally and negative results for B[a]P-induced tumors when the agent was given in the diet. In a study using ICR/Ha mice, the lung tumor multiplicity was reduced by 64% when oral oltipraz doses (500 mg/kg body weight) were given 48 h prior to each of four weekly oral 3.0 mg/kg B[a]P doses (26). Tumor inhibition was not seen when strain
A/J mice were fed oltipraz in the diet at 180 or 360 mg/kg diet 2 days before and continually after a single (i.p.) dose of B[a]P (27). In addition to the possible roles of mouse strain differences and B[a]P dosing route and schedule differences in these conflicting results, the oltipraz dosing was considerably different. In the dietary study, the 6 week dietary MTD was determined and then 0.4 and 0.8 MTD were used in the bioassay. At the higher dose, the mice consumed ~40 mg of oltipraz per kilogram body wt per day. Thus, at the time of carcinogen dosing, the mice had received ~80 mg of oltipraz per kilogram compared with 500 mg/kg in the oral dosing study. In comparison, this inhalation study used a much lower dose (1.26 mg/kg body wt), which appeared to be highly effective in reducing the number of B[a]P-induced tumors. We hope that one of the benefits of low-dose aerosol administration of oltipraz directly to the lung would be to avert the toxicities of orally administered oltipraz, such as numbness, tingling and pain in the fingertips.

It is a striking and important result that oltipraz, thought to protect selectively through Phase II enzyme(s) induction mechanism (though there are indications from in vitro studies that it may involved in the detoxification process of B[a]P by inducing CYP1A1 and CYP1B1 (20,37), was almost equally effective when given in the post-initiation phase. We are planning to add lower doses to obtain a dose-dependent response and investigative mechanisms in this post-initiation model, as it will be more relevant to chemopreventive use in human clinical trials. In fact, another dithiolethione compound, anethole dithiolethione, has been tested in clinical trials (38) for preventing progression of precancerous lesions in former smokers with excellent outcome (>50% reduction in progression of lesions compared with the placebo group).

The development of tumors in the A/J mouse lung involves activation of the K-ras gene, which codes for a protein known to be important for cell growth control and neoplastic transformation (39). The pattern of K-ras gene mutations induced in A/J mice by B[a]P is very similar to that found in activated K-ras positive human adenocarcinomas providing support for the model’s relevance and potential utility as a research tool. In an EPA-sponsored study that included evaluation of tumors induced in the lungs of A/J mice by B[a]P, 82% of the tested tumors had K-ras mutations (40). In B[a]P-induced mouse lung tumors, all K-ras mutations were in codon 12 and the predominant mutations were transversions involving the first or second base pair (40,41). There are also two hamster respiratory tract models, which are commonly used, in chemopreventive studies. In one model, MNU induces squamous cell carcinomas in the trachea, and in the other model, tracheal tumors derived from epithelial basal cells and lung tumors derived from Clara cells and endocrine cells are induced by diethylnitrosamine (42,43). The B[a]P-induced mouse lung model complements these models by providing a second species and a tumor type with a different histogenesis. The need for multiple respiratory tract tumor models reflects the diversity of cell types lining the airways. In conclusion, the results from this study clearly demonstrate that the aerosolized oltipraz can effectively function both in the pre-initiation and post-initiation phase by inhibiting B[a]P-induced adenocarcinomas in A/J mice. However, additional studies using multiple doses of oltipraz in the post-initiation phase are necessary to determine the efficacy and mechanism of action in the cancer progression phase as it is more relevant to human clinical trials for the chemoprevention of lung cancer.

Acknowledgements

We thank Timothy Morris for excellent technical assistance and Tony Godwin for animal procedures. The financial support for this work was from the Division of Cancer Prevention, National Cancer Institute (#N01-CN-25112).

Conflict of Interest Statement: None declared.

References


Received September 9, 2005; revised March 27, 2006; accepted April 13, 2006