Inhibition of cigarette smoke-related lipophilic DNA adducts in rat tissues by dietary oltipraz

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The present study investigated the effects of dietary oltipraz on cigarette smoke-related lipophilic DNA adduct formation. Female Sprague–Dawley rats were exposed daily to sidestream cigarette smoke in a whole-body exposure chamber 6 h/day for 4 consecutive weeks. One group of rats was maintained on control diet while another group received the same diet supplemented with either a low (167 p.p.m.) or high (500 p.p.m.) dose of oltipraz, starting 1 week prior to initiation of smoke exposure until the end of the experiment. Analysis of lipophilic DNA adducts by the nuclease P1-mediated 32P-post-labeling assay showed up to five smoke-related adducts. Adduct no. 5 predominated in both the lung and the heart while adduct nos. 3 and 2 predominated in the trachea and bladder, respectively. Quantitative analysis revealed that the total adduct level was the highest in lungs (270 ± 68 adducts/10^10 nucleotides), followed by trachea (196 ± 48 adducts/10^10 nucleotides), heart (141 ± 22 adducts/10^10 nucleotides) and bladder (85 ± 16 adducts/10^10 nucleotides). High dose oltipraz treatment reduced the adduct levels in lungs and bladder by >60%, while the reduction in lungs in the low-dose group was ~35%. In trachea, the effect of low and high dietary oltipraz on smoke DNA adduction was equivocal, while smoke-related DNA adducts in the heart were minimally inhibited by high-dose oltipraz. In a repeat experiment that employed a 3-fold lower dose of cigarette smoke, oltipraz (500 p.p.m.) was found to inhibit the formation of DNA adducts in rat lungs and trachea by 80 and 65%, respectively. These data clearly demonstrate a high efficacy of oltipraz in inhibiting the formation of cigarette smoke-induced DNA adducts in the target tissues.

The role of tobacco smoke in the incidence of cancers, especially of the lung, has been well documented (1,2). Scores of carcinogenic substances have been identified in smoke and various past studies, including our own, have clearly shown the formation of DNA adducts in the lungs, heart and other tissues following inhalation exposure to cigarette smoke in both rodents and humans (1,3–5). Although many studies have examined the role of chemopreventive agents in blocking the formation of DNA adducts by prototype carcinogens e.g. aflatoxin B1, 7,12-dimethylbenz[a]anthracene etc., minimal work has been done on the chemoprevention of cigarette smoke-induced DNA adducts (5,6). The present study was designed to determine if dietary oltipraz would protect against the formation of DNA adducts in rats by cigarette smoke exposure. Oltipraz was originally developed by Rhone Poulenc Sante to treat schistosomiasis (7) but has been shown to significantly reduce the incidence of tumors in a variety of experimental tumor models (8–10). Rodent and hepatocyte culture studies have suggested that oltipraz may act by enhancing the gene expression of a variety of phase II drug metabolizing enzymes (11,12) while simultaneously inhibiting certain CYP450 isozymes (13). In the present study 32P-post-labeling assay was employed to quantify a variety of highly lipophilic DNA adducts resulting from exposure to cigarette smoke.

Diet supplemented with low (167 p.p.m.) and high (500 p.p.m.) levels of oltipraz (National Cancer Institute, Bethesda, MD) were prepared twice a week by slowly mixing the compound with powdered 4% Teklad rat chow (Harlan Teklad, Madison, WI). Mixing was performed in the dark for 1 h using a Hobart mixer (Hobart Manufacturing, Troy, OH) and the diets were stored in plastic bags at ~20°C before use in experiments for the next 3–4 days. Stability of oltipraz in the diet was confirmed by HPLC analysis.

Twelve-week-old female Sprague–Dawley rats, purchased from Harlan Sprague–Dawley (Indianapolis, IN) were exposed daily to sidestream cigarette smoke from the University of Kentucky research cigarettes (IR4F) in a whole-body exposure chamber set at ~30 mg of smoke total particulate matter (TPM*)/m^3 for 6 h/day for 4 weeks, as described previously (5). The smoke-exposed groups (8–9 rats/group) received either control diet or diet supplemented with oltipraz (167 p.p.m. and 500 p.p.m.) beginning 1 week prior to and throughout the period of smoke exposure. In a repeat study, the smoke TPM in the chamber was reduced to ~10 mg/m^3 and animals maintained on control and oltipraz (500 p.p.m.)-supplemented diets were exposed to smoke as described above. Sham groups (n = 4–5) exposed to filtered ambient air were maintained in parallel in both the studies on control diet. Exposures were carried out daily for 4 weeks and the rats were killed 15 h after the last smoke exposure by a lethal dose of pentobarbital. Tissues were collected and DNA was isolated from tissues of individual animals or from pooled tissues (e.g. bladder) of 2–3 animals employing a rapid solvent extraction procedure described previously (14).

Analysis of lipophilic DNA adducts was performed by 32P-post-labeling assay after enrichment of adducts by nuclease P1 method (15), with minor modifications (14). Briefly, DNA (~17 µg) was digested with micrococcal nuclease and spleen phosphodiesterase and adducts were enriched with nuclease P1. The enriched adducts were labeled with T4 polynucleotide kinase in the presence of molar excess of [γ-32P]ATP (100 µCi; <2 µM; ~3000 Ci/mmol) prepared from carrier-free 32P (ICN Pharmaceuticals, Costa Mesa, CA) (14) and resolved by multidirectional PEI-cellulose TLC using the following

*Abbreviation: TPM, total particulate matter.

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solvents: 1 M sodium phosphate, pH 6.0 in D1 (opposite D3 direction); 4 M lithium formate/7 M urea, pH 3.5 in D3; isopropanol:4 M NH₄OH (1.2:1) in D4; and 1 M sodium phosphate, pH 6.0 in D5. The developments in D4 and D5 were run onto a 2.5 and 4 cm Whatman no. 1 wick, respectively; D1 development was performed with 5 cm of Whatman no. 17 wick attached to the top of the sheet. Adducts were detected by screen-enhanced autoradiography at –80°C for 24 h using Kodak XAR-5 film. The data were analyzed by the analysis of variance followed by Bonferroni procedure (16) and the criterion for significance of the difference between the means (± SD) was P < 0.05.

Effective exposure of animals to cigarette smoke was confirmed by the elevated levels of blood carboxyhemoglobin and plasma cotinine (data not shown). Smoke-exposed animals showed slight body weight loss (<10%) during the first 2 weeks but recovered and had weights comparable with the sham group by the end of the studies. No significant differences in the body weights of rats receiving oltipraz-supplemented diets or control diet were observed. Analysis of oltipraz in the diet did not reveal any significant loss during storage of low- and high-dose diets (≥95% recovery after a 3 day storage).

Smoke exposure resulted in 4–5 DNA adducts in the lungs, heart, trachea and bladder (Figure 1). Smoke-exposed rats showed one major (no. 5) and several minor adducts in the lungs and heart. Consistent with our earlier observations (5), adduct no. 3 predominated in trachea and adduct no. 2 in the bladder; however, additional minor adducts were also seen. Significantly lower levels of adducts were also present in the sham-treated rat tissues. Radioactivity measurements revealed the highest levels of adducts in lungs (270 ± 68 adducts/10¹⁰ nucleotides), followed by trachea (196 ± 48 adducts/10¹⁰ nucleotides), heart (141 ± 22 adducts/10¹⁰ nucleotides) and bladder (85 ± 16 adducts/10¹⁰ nucleotides) (Figure 2).

Intervention with dietary oltipraz (500 p.p.m.) inhibited all the smoke-related DNA adducts equally and substantially (∼60%) in both lungs (P < 0.01) and bladder (P < 0.05) (Figures 1 and 2). Oltipraz treatment also significantly reduced the smoke-related DNA adducts in the heart (30%, P < 0.01) but the inhibition of adducts in trachea was ~10%. Low-dose

**Fig. 1.** Representative [³²P]DNA adduct maps from lung, trachea, heart and bladder of sidestream cigarette smoke-exposed rats given control diet (B, E, H and K) or diet supplemented with 500 p.p.m. of oltipraz (C, F, I and L); adduct maps for low dose oltipraz group not shown. Animals were exposed daily for 4 weeks to sidestream cigarette smoke (30 mg TPM/m³). (A, D, G and J) Maps from sham-treated rats. DNA adducts (from 15 µg) were enriched by nuclease P1, [³²P]-labeled using carrier-free [γ³²P]ATP (~3000 Ci/mmol; 100 µCi; ~2 µM) and separated by multidirectional PEI-cellulose TLC using the solvents as described in the text. Adducts were detected by screen enhanced autoradiography at –80°C for 24 h using Kodak XAR-5 films.

**Fig. 2.** Comparison of DNA adduct levels in (A) lung, (B) trachea, (C) heart and (D) bladder of rats exposed to cigarette smoke and low (167 p.p.m.; L) or high (500 p.p.m.; H) dose oltipraz (Olt.). Mean values were calculated from 8–9 animals, except for sham group in which the mean is from 4–5 animals and represented as mean ± SD. In (D), values in sham, smoke alone and smoke plus oltipraz-supplemented diet represent the average from two pools by pooling the bladder from either two or three animals.
oltipraz (167 p.p.m.) significantly inhibited smoke-related DNA adducts in the lungs by ~35% ($P < 0.01$) but was ineffective in the trachea.

In a repeat experiment, rats fed a high dose of oltipraz (500 p.p.m.) were exposed to reduced level of cigarette smoke particulates (~3-fold), while other conditions remained the same. The results showed that the smoke-related DNA adducts in the lungs and trachea were qualitatively similar to those seen in the first experiment (not shown). Quantitative analysis showed that the adduct levels in this experiment were 138 ± 21 per $10^{10}$ nucleotides and 60 ± 15 per $10^{10}$ nucleotides in the lungs and trachea, respectively. Animals fed the high oltipraz diet showed a reduction of >80 and ~65% in the levels of smoke-related DNA adducts in the lungs and trachea, respectively ($P < 0.05$). Heart and bladder were not analyzed in this study.

These results suggest that oltipraz and related compounds, which are known to act by inducing various phase II conjugating enzymes (11,12), may provide chemopreventive effects against smoke-induced DNA adducts. Furthermore, the findings in lung and trachea that oltipraz is more effective against low-dose exposures of cigarette smoke suggest that higher smoke exposures may overwhelm the protective effects of oltipraz. Interestingly, bladder, which is a distant tissue from direct smoke exposure, showed a strong (>60%) inhibition of adducts by oltipraz (500 p.p.m.).

It should be noted that cigarette smoke is a highly complex mixture of chemicals containing carcinogens, tumor promoters and inhibitors. Three carcinogens, benzo(a)pyrene, 4-(methyl)nitrosamine-1-(3-pyridyl)-1-butanone and 4-aminobiphenyl, which are present in cigarette smoke, have been extensively studied, with respect to their ability to form DNA adducts; however, their exact contribution to smoke-induced lung cancer is still not known. Based on our past and present results, it appears unlikely that the adducts detected by $^{32}$P-post-labeling assay correspond to any of the adducts formed by the above three carcinogens (3,5,17). Thus, the identity of smoke-related DNA adducts is not established. Based on the TLC solvents and the identity of the products observed in the present study strongly support the further development of this specific model employing the most relevant of human carcinogens as well as more detailed studies with other chemopreventive agents.

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