Aerosolized 3-Bromopyruvate Inhibits Lung Tumorigenesis without Causing Liver Toxicity

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Abstract

3-Bromopyruvate, an alkylating agent and a well-known inhibitor of energy metabolism, has been proposed as a specific anticancer agent. However, the chemopreventive effect of 3-bromopyruvate in lung tumorigenesis has not been tested. In this study, we investigated the chemopreventive activity of 3-bromopyruvate in a mouse lung tumor model. Benzo(a)pyrene was used to induce lung tumors, and 3-bromopyruvate was administered by oral gavage to female A/J mice. We found that 3-bromopyruvate significantly decreased tumor multiplicity and tumor load by 58% and 83%, respectively, at a dose of 20 mg/kg body weight by gavage. Due to the known liver toxicity of 3-bromopyruvate in animal models given large doses of 3-bromopyruvate, confirmed in this study, we decided to test the chemopreventive activity of aerosolized 3-bromopyruvate in the same lung tumor model. As expected, aerosolized 3-bromopyruvate similarly significantly decreased tumor multiplicity and tumor load by 49% and 80%, respectively, at a dose of 10 mg/mL by inhalation. Interestingly, the efficacy of aerosolized 3-bromopyruvate did not accompany any liver toxicity indicating that it is a safer route of administering this compound. Treatment with 3-bromopyruvate increased immunohistochemical staining for cleaved caspase-3, suggesting that the lung tumor inhibitory effects of 3-bromopyruvate were through induction of apoptosis. 3-Bromopyruvate also dissociated hexokinase II from mitochondria, reduced hexokinase activity, and blocked energy metabolism in cancer cells, finally triggered cancer cell death and induced apoptosis through caspase-3, and PARP in human lung cancer cell line. The ability of 3-bromopyruvate to inhibit mouse lung tumorigenesis, in part through induction of apoptosis, merits further investigation of this compound as a chemopreventive agent for human lung cancer.

Introduction

Lung cancer is the leading cause of cancer-related deaths in the United States and continues to be the most common fatal cancer (1). The response and survival rates of patients have remained low (2), with few options for high-risk populations in primary prevention of cancer recurrence. The late presentation of lung cancer symptoms is a major reason for the lack of progress in treatment of this disease. Thus, there is an urgent need to develop efficient strategies to treat lung cancer. Chemoprevention, centered around the administration of natural or synthetic compounds to inhibit, delay, or reverse the process of carcinogenesis, could be an effective approach to reduce the risk of developing or decreasing the incidence of lung cancer (3, 4) especially in the high-risk population. Chemoprevention is considered to be an important approach to decrease lung cancer (5).

In the 1930’s, Warburg first observed that cancers consistently use more glucose and produce more lactic acid than normal tissue (6). He proposed this alteration as a fundamental metabolic change during malignant transformation or "the origin of cancer cells." Cancer cells rely on glucose transporters for glucose uptake and subsequent glycolysis through hexokinase (HK) and other glycolytic enzymes for their survival and proliferation. Thus, selective inhibition of glucose uptake and/or glycolysis rate in cancer cells provides another novel therapeutic target in cancer (7).

HK is the initial and rate-limiting enzyme in the glycolytic pathway in all mammalian tissues including the lung (8). 3-Bromopyruvate (3-BrPA) is an alkylating agent and a potential inhibitor of HK II, which effectively inhibits glycolysis. Out of the 4 major isozymes, HK II constitutes the principal regulated isozyme in many cell types and is increased in many cancers. Recent studies have revealed that 3-BrPA is an
effective anticancer agent in different cancer cell lines and animal tumor models (9–12).

In this study, we determined the effect of 3-BrPA on benzo(a)pyrene [B(a)P]-induced lung tumorigenesis by oral gavage or by aerosol. 3-BrPA inhibited B(a)P-induced lung tumorigenesis by both treatments. Immunohistochemical characterization of lung tumors indicated that 3-BrPA increased cleaved caspase-3 in treated mice. The proapoptotic effects of 3-BrPA were also observed on human cancer cells in vitro. These data support further investigation of 3-BrPA as a potential lung cancer chemopreventive agent.

Materials and Methods

Reagents and animals

3-BrPA, B(a)P (99% pure), tricaprylin, and MTT were purchased from Sigma Chemical Co. B(a)P was prepared immediately before use in animal bioassays. For Western blotting analysis, antibodies against HK II, caspase-3, cleaved caspase-3 (Asp175), PARP, and cleaved PARP (Asp214), were purchased from Cell Signaling Technologies. Female A/J mice at 6 weeks of age were obtained from Jackson Laboratories.

Chemopreventive studies

Two animal studies were carried out (Fig. 1). In experiment 1, mice were given a single i.p. injection of B(a)P at 100 mg/kg body weight in tricaprylin. Mice were then randomized into 3 groups with 10 mice per group: (i) control group; (ii) 3-BrPA low-dose group (10 mg/kg; BW), randomized into 3 groups with 10 mice per group: (i) 100 mg/kg body weight in tricaprylin. Mice were then treated for 20 weeks. (iii) 3-BrPA high-dose group (20 mg/kg. BW). 3-BrPA was dissolved in PBS and adjusted to pH 7.4 with NaOH just before gavage administration, intragastrically was administered once a day 5 times a week for the duration of the study. Control animals were treated with PBS throughout the study. The treatment was begun 2 weeks after the B(a)P injection and continued for 20 weeks.

In experiment 2, female A/J mice at 6 weeks of age from Jackson laboratories were given a single i.p. injection of B(a)P at 100 mg/kg body weight in tricaprylin. Two weeks after B(a)P injection, mice were randomized into 4 groups with 12 mice per group for aerosol exposure: (i) air control group; (ii) vehicle control group [dimethyl sulfoxide (DMSO): EtOH = 20:80]; (iii) 3-BrPA low-dose group (2 mg/mL); (iv) 3-BrPA high-dose group (10 mg/mL). Powdered 3-BrPA was dissolved in a 20% DMSO:EtOH solution to give 3-BrPA concentration 2 and 10 mg/mL. The solution was freshly prepared every day. Solution formulations were atomized into droplets by atomizer. Aerosol flow was then passed through 2 scrubbers with activated carbon to remove ethanol and DMSO. The resulting dry aerosol flow with only desired chemicals was then introduced into the nose-only exposure chamber from the top inlet. Effluent aerosol was discharged from an opening at the bottom of the chamber. This formulation was administered once a day 5 times a week. Vehicle controls were exposed to 20% DMSO:EtOH solution. All formulations were prepared immediately before dosing. All groups were treated for 8 minutes and continued for 20 weeks.

Animals were housed with wood chip bedding in environmentally controlled, clean air room with a 12-hour light–dark cycle and a relative humidity of 50%. Drinking water and diet were supplied ad libitum. The study was approved by the Washington University’s Institutional Animal Care and Use Committee.

For both experiments, body weight was recorded weekly (Supplementary Fig. S1). Mice were euthanized by CO2 asphyxiation. Lungs of each mouse were fixed in Tellyesniczky’s solution (90% ethanol (70% v/v), 5% glacial acetic acid, and 5% formalin) overnight then stored in 70% ethanol (13). The fixed lungs were evaluated under a dissecting microscope to obtain surface tumor count and individual tumor diameter. Tumor volume was calculated based on the following formula: 

$$V = \frac{4}{3}\pi r^3$$

(14). The total tumor volume in each mouse was calculated from the sum of all tumors. Tumor load was determined by averaging the total tumor volume of each mouse in each group.

The size distribution of the aerosol was determined by Scanning Mobility Particle Sizer spectrometer, which includes an Electrostatic Classifier (TSI model 3080), a Differential Mobility Analyzer (TSI model 3081), and a Condensation Particle Counter (TSI model 3025). Geometric median diameters, mass median aerodynamic diameter (MMAD), geometric SD were obtained.

In situ immunohistochemical evaluation of apoptosis in lung tumor tissue

Five lungs from each group of A/J mice were analyzed to evaluate activated caspase-3 staining in lung tissues. Immunohistochemistry was carried out on lung tissue sections using specific antibodies to detect localization and quantify...
the number of positive cells (15). Antibodies against activated caspase-3 (Biocare) were used at a 1:400 dilution. In brief, all slides were deparaffinized in xylene and rehydrated in gradients of ethanol. Microwave antigen retrieval was carried out for 20 minutes in citrate buffer, pH 5.0 to 6.0. Primary antibody was diluted in DaVinci Green (BioCare) and incubated at 4°C overnight. Secondary antibody diluted in PBS Tween-20 (PBST) and SA-HRP (HRP, horseradish peroxidase; 1:800) was then applied to the sections. Negative control slides were processed in the absence of the primary antibody. Manual counting of labeled and total cells in control slides were processed in the absence of the primary antibody. Manual counting of labeled and total cells in high-powered (×400) fields of tumor tissue was conducted.

Cell viability assay

The cytotoxicity of 3-BrPA was assessed with the MTT method, according to standard protocols. Briefly, human lung cancer H1299, A549, H23 cells were obtained from American Type Culture Collection and were maintained in RPMI-1640 medium supplemented with 10% FBS (Gibco), 100 U/mL penicillin, and 100 mg/mL streptomycin at 37°C with 5% CO2. Cells were seeded onto 96-well tissue culture plates at 2,000 cells per well. Twenty-four hours after seeding, cells were exposed to different concentrations of 3-BrPA as indicated for 24 or 48 hours, while that of the control group was replaced with fresh medium. The 3-BrPA solutions were prepared in RPMI-1640 medium, and then they were adjusted to pH 7.4 with NaOH. The solutions were sterilized with 0.2 μm filter unit (Millipore). MTT (0.5 mg/mL) was added after the exposure period. The formazan crystals that formed were dissolved in DMSO after 4-hour incubation, and the absorbance was measured at 595 nm and 655 nm by Infinite M200 Pro plate reader (Tecan). All assays were conducted in triplicate.

Isolation of mitochondria and protein fractionation

Mitochondrial and cytosolic fractions were isolated by Mitochondria/Cytosol Fractionation Kit (Abcam) according to the manufacturer’s instructions (16). Briefly, after 3-BrPA treatment, A549, H1299, and H23 cells were harvested, washed once with ice-cold PBS, and resuspended with 1.0 mL of 1× cytosol extraction buffer Mix containing dithiothreitol (DTT) and protease inhibitors. After incubating on ice for 10 minutes, the cell suspension was homogenized with ice-cold glass homogenizer for 30 to 50 times. The samples were centrifuged at 3,000 rpm at 4°C for 10 minutes. The supernatants were centrifuged again at 13,000 rpm for 30 minutes to separate the mitochondrial fraction (pellets) and the cytosolic fraction (supernatants). The mitochondria pellet was washed once with the isolation buffer, and then lysed in mitochondria extraction buffer containing DTT and protease inhibitors.

HK assay

The activity of HK was measured as previously described (12, 17) with modification. After pretreatment with 3-BrPA, cells were washed with cold PBS, then lysed using the following buffer: 50 mmol/L potassium phosphate, 2 mmol/L DTT, 2 mmol/L EDTA, and 20 mmol/L sodium fluoride. After the cells were harvested, the cell homogenate was incubated on ice for 30 minutes, followed by centrifugation at 1,000 × g at 4°C for 10 minutes. Approximately 10 μL of freshly lysed cell supernatant was added to 200 μL of HK reaction buffer [100 mmol/L Tris-HCl, pH 8.0, 0.5 mmol/L EDTA, 1.25 mmol/L ATP, 10 mmol/L MgCl2, 2 mmol/L glucose, 0.1 mmol/L NADP, and 0.2 U/mL of G6PD (Sigma-Aldrich)]. HK activity was determined by following the G6P-dependent conversion of NADP to NADPH spectrophotometrically at 340 nm at 37°C. One activity unit is defined as micromoles of NADPH per milliliter per minute at 37°C.

Cell apoptosis analysis

3-BrPA–induced apoptosis and necrosis was analyzed by flow cytometry with BD Pharmingen PE Annexin V Apoptosis Detection Kit (BD Biosciences) according to the manufacturer’s instructions. Briefly, H1299 cells treated with 50 and 100 μmol/L 3-BrPA for 24 hours were collected. After washing twice with cold PBS, cells were resuspended in 0.1 mL of ×1 binding buffer containing Annexin V–phycoerythrin (PE) and 7-amino-actinomycin (7-AAD). Cell preparations were incubated on ice for 15 minutes in the dark. The samples were then added to ice-cold binding buffer and analyzed by flow cytometry using a FACScalibur detector (BD Biosciences) according to the protocol provided by the manufacturer.

Antibodies and Western blotting

Mitochondria and cytosol were separated by PAGE, transferred to a polyvinylidene difluoride membrane and blotted with primary antibodies against HK II, caspase-3, cleaved caspase-3 (Asp175), PARP, and cleaved PARP (Asp214). Signals were visualized by the ECL Western Blotting Analysis System.

Liver toxicology of 3-BrPA in A/J mice

We carried out liver toxicology studies to comparatively evaluate potential liver toxicity in mice treated with 3-BrPA for 8 weeks by gavage and aerosol routes. A/J mice (6 weeks old) were obtained from Jackson Laboratories. For gavage treatment, the vehicle control group and gavage treatment group (20, 40, 60, and 80 mg/kg.bw) were treated 5 times every week and continued for 8 weeks. For aerosol treatment, mice were treated with 10 and 30 mg/mL 3-BrPA 5 times and continued for 8 weeks. Body weights were monitored weekly for the duration of the studies, and mice showing 20% or more of body weight loss were euthanized by CO2 asphyxiation. All food and water were available ad libitum. After 8 weeks of treatment, serum was collected for alanine transaminase (ALT) and aspartate aminotransferase (AST) assay, and mice were euthanized by CO2 asphyxiation. Serum ALT and AST determinations were carried out according to validated laboratory protocols using an Ortho
Clinical Diagnostic Vitros Fusion 5.1 analyzer. Liver tissue samples were fixed in 10% buffered zinc-formalin and embedded in paraffin. Three micrometer sections were stained with hematoxylin and eosin (H&E) and histologically evaluated.

**Statistical analysis**

The data on tumor multiplicity, tumor load, apoptotic index were analyzed by 2-tailed Student t test. *, *P < 0.05; **, *P < 0.01; ***, *P < 0.001.

**Results**

**Inhibitory effect of 3-BrPA on lung tumor multiplicity and tumor load in B(a)P-induced A/J mice by oral gavage**

In experiment 1, we determined the effect of 3-BrPA on B(a)P-induced lung tumorigenesis by oral gavage. Lung tumor incidence was 100% in each group. B(a)P-induced an average of 8.7 ± 1.6 tumors per mouse in the control group, and tumor load was 3.1 ± 0.6 mm³. Mice treated with 10 and 20 mg/kg BW 3-BrPA showed a significant decrease in both tumor multiplicity (47% inhibition, 4.6 ± 1.2 tumors; 58% inhibition, 3.6 ± 1.3 tumors) and tumor load (73% inhibition, 0.8 ± 0.3 mm³; 83% inhibition, 0.5 ± 0.3 mm³) when compared with the control group (Fig. 2A, 2B).

**3-BrPA leads to mild liver toxicity in high-dose 3-BrPA by gavage indicated by increased ALT and AST levels**

Considering the possible liver toxicity that 3-BrPA may have, we carried out an 8-week liver toxicity study in female A/J mice treated by gavage or aerosol. Mice given 80 mg/kg bw of 3-BrPA by gavage were terminated within the first 2 weeks due to body weight loss (>20%). Mice in other gavage 3-BrPA dosage groups and in all control and inhalation dosage groups tolerated treatment well, without significant difference in body weights (data not shown) between these groups.

Serum ALT and AST levels were measured in the mice after treatment with 3-BrPA for 8 and 20 weeks by gavage or aerosol (Fig. 3). The results revealed no significant differences between the aerosol control groups and aerosolized 3-BrPA groups. However, serum levels of these enzymes were significantly increased in the gavage 3-BrPA treatment group compared with the control mice, indicative of liver damage induced by oral gavage treatment with 3-BrPA.

Histologic changes of liver treated with 3-BrPA for 8 weeks seen in the lower dosage gavage groups and in all aerosol groups were minimal to absent. (Supplementary Fig. S2B). The histologic sections of the liver from the high-dose gavage group (60 mg/kg bw) showed a moderate increase in size and number of lymphohistiocytic aggregates associated with focal pyknosis of adjacent hepatocytes (Supplementary Fig. S2C and S2D) compared with gavage control group (Supplementary Fig. S2A). This change was not accompanied by fibrosis, indicating that the damage incurred within the time frame of our studies was reversible. Histologic changes of liver treated with 3-BrPA for 20 weeks were also mild (Supplementary Fig. S3A–S3C).

**Inhibitory effect of 3-BrPA on lung tumor multiplicity and tumor load in B(a)P-induced A/J mice by inhalation**

We next determined the effect of aerosolized 3-BrPA on lung tumorigenesis induced by B(a)P in A/J mice. The aerodynamic typical particle size distribution of nebulized 2 and 10 mg/mL 3-BrPA was as follows: The geometric median diameter was 0.033 and 0.037 μm and geometric...
SD was 1.8. The MMAD of 3-BrPA was approximately 0.2 μm. In the solvent control group, tumor multiplicity was 6.3 ± 1.0, and tumor load was 2.2 ± 0.5 mm³, there is no significant difference on tumor multiplicity and tumor load compared with air control group. When treated with 3-BrPA by inhalation, tumor multiplicity and tumor load decreased significantly. Mice treated with 2 and 10 mg/mL 3-BrPA had a significant decrease in both tumor multiplicity (31% inhibition, 4.4 ± 0.9 tumors; 49% inhibition, 3.3 ± 0.9 tumors) and tumor load (58% inhibition, 0.9 ± 0.4 mm³; 80% inhibition, 0.5 ± 0.3 mm³) when compared with the solvent control group. Administration of 3-BrPA did not have a significant effect on body weight in either the gavage or aerosol protocols (Supplementary Fig. S1).

3-BrPA induced cell apoptosis in B(a)P-induced lung tumorigenesis

Caspases are an evolutionary conserved family of cysteine proteases that are involved in apoptosis (18). Many chemopreventive trials have used caspase-3 as a biomarker of apoptosis (19). To determine the extent of apoptosis in lung tumors from treated and untreated mice, lung sections were stained with cleaved caspase-3 antibody. There was a significant increase in the number of cleaved caspase-3–positive cells and in the lungs of mice given aerosolized 3-BrPA compared with untreated mice (Fig. 4). 3-BrPA treatment increased the percentage of caspase-3–positive cells from 0.6% in the control group, to 4.5% in the 10 mg/mL 3-BrPA group (7-fold compared with control, P < 0.001). Mice treated with 3-BrPA by gavage showed similar effect (data not shown). These results indicate that treatment with inhaled 3-BrPA increased the apoptotic index. In our study, we also carried out immunohistochemistry of Ki-67 on lung tissues and did not observe significant changes (data not shown).

3-BrPA decreased A549, H1299, and H23 cells viability and HKII expression in mitochondria

To investigate the possible mechanism that 3-BrPA acts on, human non–small lung cancer cells A549, H1299, and H23 were exposed to different concentrations of 3-BrPA and cell viability measured by MTT assay. We observed that treatment of these cells with 3-BrPA significantly decreased cell viability in a dose- and time-dependent manner (Fig. 5A). But different cell lines have different sensitivity for 3-BrPA, among all the 3 cell lines, A549 is the most resistant cell line and H23 is the most sensitive cell lines. As 3-BrPA is a potential HKII inhibitor, we also checked the HKII expression in mitochondrial treated with 3-BrPA. Mitochondria-enriched fractions from 3-BrPA–treated cancer cells were isolated, and the expression of HKII was analyzed by Western blot analysis. As shown in Fig. 5B, mitochondria isolated from H1299 and H23 cells treated with 3-BrPA exhibited a significant loss of HKII, indicating a possible quick release of HKII from mitochondria after 3-BrPA treatment. But for A549 cells, the basal level of HKII expression was very low; we did not detect any change on HKII expression after 3-BrPA treatment.

3-BrPA decreased HK activity, reduced ATP production, and induced cell apoptosis in H1299 cells

To further investigate whether 3-BrPA regulates HK to inhibit lung tumor cells growth, we next evaluated the effect of 3-BrPA on HK activity in all 3 cell lines. As shown in Fig. 6A, 3-BrPA significantly reduced HK activity in a time- and dose-dependent manner in all 3 cell lines. To clarify the role
of glycolysis in ATP production in lung cancer cells, we measured the intracellular levels of ATP upon treatment with 3-BrPA. As shown in Fig. 6B, both H1299 and H23 exhibited a significant dose- and time-dependent decrease in cellular ATP levels starting at 30 minutes exposure to 3-BrPA, confirming its ability to block energy metabolism in these cell line. But in A549, the reduced HKII activity did not suppress ATP production.

In our in vivo study, we noticed a significant increase in cleaved caspase-3 expression in mouse lungs after treatment with 3-BrPA, confirming its ability to block energy metabolism in lung cancer cells. We measured the intracellular levels of ATP upon treatment with 3-BrPA. As shown in Fig. 6B, both H1299 and H23 exhibited a significant dose- and time-dependent decrease in cellular ATP levels starting at 30 minutes exposure to 3-BrPA, confirming its ability to block energy metabolism in these cell line. But in A549, the reduced HKII activity did not suppress ATP production.

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with 3-BrPA. We next investigate whether 3-BrPA can induce apoptosis in vitro. H1299 cells incubated with different concentrations of 3-BrPA were collected and analyzed for Annexin V immunoreactivity by flow cytometry (Fig. 6C). Treatment of H1299 cells with 100 μmol/L of 3-BrPA increased the percentage of apoptotic cells from 5.6% in control group to 30.2%. Coincidentally, the percentage of end-stage apoptotic and dead cells increased from 3.9% to 21.9%. Similar with our in vivo findings, cells treated with 50 and 100 μmol/L 3-BrPA for 24 hours exhibited activation of caspase-3 (Fig. 6D), additionally, full-size PARP (116 kD) protein was also cleaved to yield an 89 kD fragment after treatment of cells with 3-BrPA. These results indicate that 3-BrPA may cause ATP depletion.
and apoptotic cell death by dissociation of HK II from mitochondria.

Discussion

In normal cells, 90% of ATP is provided by mitochondrial oxidative phosphorylation (20). In contrast, tumor cells derive most of their metabolic energy from glycolysis. Because glycolysis generates only 2 moles of ATP per mole of glucose while oxidative phosphorylation within mitochondria generates 30 ATP per glucose, tumor cells have to upregulate glycolysis to meet their energy requirements (21). Cancer cells shift their energy production from oxidative phosphorylation toward the less efficient glycolysis pathway and catabolize glucose at a higher rate than their nontransformed counterparts. This is termed the Warburg effect (22).

Because cancer cells rely heavily upon glycolysis for ATP production, inhibition of this metabolic pathway provides a potential target for cancer treatment. HK is the first enzyme in the glycolytic pathway. Out of 4 major isoforms, HK II is suggested to be the main isofrom regulating glucose metabolism in cancer cells (23, 24). In our previous study, we found the expression of HK II was upregulated in B(a)P- or NNK-induced mouse lung tumors compared with normal lung tissue (25). Based on these results, we hypothesized that 3-BrPA can inhibit B(a)P-induced lung tumorigenesis.

In this study, we evaluated the chemopreventive efficacy of 3-BrPA by 2 different administration, gavage or aerosol. We found that 3-BrPA significantly decreased tumor multiplicity and tumor load by both administration, but 3-BrPA administered by gavage cause mild liver toxicity revealed by increased ALT and AST levels, as well as the pathologic change from H&E staining. In contrast, mice treated with aerosolized 3-BrPA did not exhibit elevated ALT and AST levels. As In our study, high-dose 3-BrPA by aerosol and gavage has similar chemopreventive efficacy. Aerosol treatment shows less potential for liver toxicity than gavage delivery with similar efficacy, aerosol 3-BrPA seems to be a more ideal treatment than gavage.

Previous study showed that 3-BrPA could be a potential HKII inhibitor. In our study, we found that 3-BrPA showed different cytotoxicities in 3 lung cancer cell lines. A549, which showed more resistance for 3-BrPA in MTT assay, expresses relatively low levels of HKII compared with other 2 cell lines. 3-BrPA reduced HKII expression in mitochondrial fraction rapidly, indicating a quick release of HKII from mitochondria, which results in the dramatically reduction of HK activity. The present data also showed that a quick depletion of ATP in H1299 and H23 cell lines, but not in A549 cells.

Induction of apoptosis is an important mechanism whereby chemopreventive agents can suppress tumorigenesis (26). Previous studies have suggested that 3-BrPA can induce apoptosis in variety types of cancer cells (11, 27, 28). In our in vitro study, we observed that 3-BrPA increased staining by caspase-3 immunohistochemistry in lung tumor tissues from 3-BrPA–treated mice, consistent with a proapoptotic effect. Our data thus suggest that 3-BrPA can promote apoptosis within mouse lung tumors, and that these mechanisms are likely to contribute to the observed chemopreventive effect. The proapoptotic effect of 3-BrPA was further supported by our in vitro data that show increased Annexin V staining and caspase-3 and PARP cleavage following treatment of H1299 lung adenocarcinoma cells with 3-BrPA.

In conclusion, this study shows that aerosolized 3-BrPA induced a proapoptotic effect and therefore inhibits B(a)P-induced lung tumorigenesis in A/J mice without causing weight loss or any other observable side effects. Furthermore, we have shown that 3-BrPA functions as an HKII inhibitor in human NSCLC cell lines, which dissociated HKII from mitochondria, reduced HK activity, and blocked energy metabolism in cancer cells, finally triggered cancer cell death and apoptosis. Therefore, our results suggest that inhibition of glycolysis is a promising potential strategy for inhibition of lung cancer and that this approach may be useful for the treatment and prevention of human lung cancer. Importantly, this study shows that delivery of 3-BrPA by aerosol is an effective means of limiting systemic toxicity while retaining efficacy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: Q. Zhang, R.A. Lubet, Y. Wang, M. You
Development of methodology: Q. Zhang, J. Pan, M. You
Acquisition of data: Q. Zhang, J. Pan, P.E. North, S. Yang
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Writing, review, and/or revision of the manuscript: Q. Zhang, J. Pan, R.A. Lubet, Y. Wang, M. You
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