The Second-Generation PGI2 Analogue Treprostinil Fails to Chemoprevent Tumors in a Murine Lung Adenocarcinoma Model

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Abstract

Prostacyclin (prostaglandin I2, PGI2) overproduction in FVB/N mice prevents the formation of carcinogen and tobacco smoke-induced adenomas, and administration of the oral prostacyclin analogue iloprost to wild-type mice also prevented carcinogen-induced mouse lung adenoma formation. Former smokers taking oral iloprost showed improved bronchial dysplasia histology compared with placebo. Next-generation oral prostacyclin analogues, like treprostinil, were developed for the treatment of pulmonary arterial hypertension (PAH). On the basis of our prior studies with iloprost, we performed preclinical studies examining the ability of treprostinil to chemoprevent urethane-induced murine lung adenocarcinoma. We determined the MTD in chow (prior studies had delivered treprostinil by gavage), and this dose produced serum levels in the experimental animals similar to those found in PAH patients treated with treprostinil. We then examined the chemopreventive efficacy of treprostinil exposure initiated both before (1 week) and after (6 weeks) urethane exposure to better model chemoprevention studies conducted in former smokers. Neither of these dosing strategies prevented murine lung cancer; however, we did detect changes in pulmonary inflammatory cell infiltrate and expression of CXCR4 (a chemokine receptor previously shown to increase in response to treprostinil exposure) in tumor-bearing, treprostinil-treated animals, indicating that the drug was bioavailable. One potential explanation stems from iloprost and treprostinil differentially activating cell surface prostaglandin receptors and intracellular peroxisome proliferator-activated receptors. When murine lung tumor cells were treated with treprostinil, their proliferation rate increased; in contrast, iloprost had no effect on proliferation. Future investigations comparing these two agents will provide insight into iloprost’s chemopreventive mechanisms.

Cancer Prev Res; 10(11); 671–9. ©2017 AACR.

Introduction

Lung cancer accounts for the most cancer-related deaths across all ethnicities and genders in the United States and worldwide (1). The 5-year survival rate for lung cancer has gradually increased from 9% to just 16% in the past 40 years, even with the advent of targeted therapies (2). Lung cancer is usually diagnosed at an advanced stage, which contributes to its high mortality rate. The greatest potential for improving the poor survival rate may lie with earlier detection and more effective prevention strategies. A 20% improvement in lung cancer mortality was seen in the National Lung Screening trial with annual CT scan surveillance, and this led to endorsement of lung cancer screening by many organizations in addition to improved early detection with low-dose CT scans, chemoprevention is an appealing approach to reducing nonsmall cell lung cancer (NSCLC) mortality because we can readily identify high-risk populations and use regression of premalignant lesions (ground glass opacities and nodules for adenocarcinoma and endobronchial dysplasias for squamous cell carcinoma) as an intermediate endpoint. Many previous NSCLC chemoprevention trials were not based on robust preclinical data and used cancer diagnosis as the primary endpoint. This resulted in large, costly trials, which were generally unsuccessful (4–6) and reduced scientific enthusiasm for future lung cancer chemoprevention trials. Newer trials focusing on prevention of progression to malignant disease could potentially reduce lung cancer mortality.

We previously demonstrated that selective pulmonary production of prostacyclin (PGI2), a product of the arachidonic acid pathway targeted in the treatment of pulmonary arterial hypertension (PAH), prevented lung tumorigenesis in multiple murine lung cancer models. Mice engineered to overexpress prostacyclin synthase (PGIS) under control of the surfactant protein C promoter produce increased lung prostacyclin and develop fewer tumors in response to chemical carcinogens or tobacco smoke than do their wild-type littermates (7, 8). Iloprost, an oral prostacyclin analogue, also prevents carcinogen (urethane)-induced lung tumor formation in mice (9). In addition, iloprost inhibits anchorage-independent growth of NSCLC cell lines and activates peroxisome proliferator-activated receptor gamma (PPARγ), a known inhibitor of lung cancer growth (10, 11). These preclinical trials...
findings led to a phase II clinical trial in which high-risk current and former smokers were randomized to either placebo or oral iloprost for 6 months. Comparison of endobronchial dysplasia, a precursor of lung squamous cell carcinoma, in baseline and follow-up lung biopsies demonstrated that former smokers receiving iloprost experienced significant improvement in endobronchial dysplasia compared with those that received placebo (12). No improvement in dysplasia was seen in current smokers. Former smokers are currently being recruited to a trial (NCT02237138) testing whether inhaled iloprost also results in lesion regression.

In an effort to build on the success of the positive phase II human trial with oral iloprost and expand the pool of potential chemopreventive agents, we investigated the chemopreventive efficacy of treprostinil. Oral iloprost is no longer manufactured, and treprostinil is a next-generation prostacyclin analogue with multiple delivery formulations and a longer half-life than iloprost. Treprostinil is currently used for PAH treatment (13), and subcutaneous, intravenous, and oral formulations are FDA approved in the United States for treatment of patients with WHO group I PAH with NYHA functional class II–IV symptoms, whereas the inhaled form is approved for patients with functional class III and IV symptoms (14). More recently, oral treprostinil exhibited clinical efficacy in PAH as add on therapy, and adverse safety events were not associated with the oral formulation (15). In addition, treprostinil inhibits TGFβ and NFκB signaling, suggesting it may promote anticancer signaling (16, 17). Given the success of other prostacyclin analogues in the chemoprevention setting and treprostinil’s improved oral bioavailability/half-life, safety, and tolerability in the treatment of PAH, we hypothesized that treprostinil would effectively prevent NSCLC. Our in vitro and in vivo experimental results instead determined that treprostinil did not prevent carcinogen-induced lung tumor formation in mice and actually enhanced proliferation of mouse lung-tumor–derived cells.

Materials and Methods

Animal studies

Female FVB/N mice (4–6 weeks of age) were purchased from Harlan Laboratories and housed in microisolator cages at the Denver Veterans Administration Medical Center (DVAMC) Veterinary Medicine Unit according to a protocol approved by the DVAMC Institution of Animal Care and Use Committee in accordance with the NIH’s Guide for the Care and Use of Laboratory Animals. Mice were maintained on hardwood bedding with a 12-hour light/dark cycle; food and water were provided ad libitum.

MTD determination

Previously, mice were given treprostinil via daily gavage, so a dietary treprostinil MTD was determined prior to performing tumorigenesis studies. Twenty female FVB/N mice (5 mice/group) were fed AIN-76A chow containing 7.2% (14.4 mg/kg/day), 14.4% (28.8 mg/kg/day), or 36.0% (72 mg/kg/day) treprostinil or control AIN-76A chow (modified to contain similar maltodextrin levels to drug containing chow) for 3 weeks. Treprostinil was graciously provided by United Therapeutics and integrated into a chow formulation by LabDiet. Bacon scent was added to all chow formulations to stimulate appetite. Animal weights and activity levels were observed and recorded twice weekly. Surviving experimental mice were sacrificed after 21 days of special diet. Animals with excessive weight loss (>15% of their beginning weight) were removed from the study and euthanized.

Determination of bronchoalveolar inflammatory cell content and prostaglandin levels

Bronchoalveolar lavage (BAL) was performed as described previously (18) to quantify inflammatory cells. BAL cells were harvested by centrifugation, counted, and stained with Wright stain prior to differential counting. Prostaglandin E2 (PGE2) and 6-keto PGF1α, the stable metabolite of prostaglandin I2 (PGI2), in BAL (BLCF) were measured by ELISA according to the manufacturer's instructions (Cayman Chemicals: 500141, 515211).

Treprostinil chemoprevention study

Female FVB/N mice (8–10 weeks of age) were divided into 5 groups and fed the appropriate chow for 1 week prior to intraperitoneal injection with either 1 mg/g urethane (Sigma: U2500) or 0.9% saline vehicle. Group sizes were determined using mean and variation of tumor multiplicity from previous experiments to detect 30% differences between groups (power = 0.9). Group 1 mice (n = 10) were fed control chow followed by saline injection; group 2 mice (n = 10), treprostinil containing chow (28.8 mg/kg/d) followed by saline injection; group 3 (n = 15), control chow followed by urethane (1 mg/g body weight) injection; group 4 mice (n = 15) treprostinil followed by urethane injection 1 week later [early treatment (ET) group]; and group 5 mice (n = 15) fed control chow were injected with urethane, and switched to treprostinil chow 6 weeks after urethane exposure [late treatment (LT) group]. Eighteen weeks after urethane injection, mice were sacrificed by lethal pentobarbital injection, and plasma was collected by cardiac puncture. BALF was collected and differential cell counts were performed. Blood was perfused from the lungs at the time of sacrifice by direct instillation of PBS through the pulmonary artery. Tumors were dissected from normal appearing tissue (uninvolved) in 10 to 12 mice/group, counted, their diameters measured, and flash frozen. The right lower lobe was digested with 1 mg/mL collagenase (Sigma: C2139) at 37°C for 25 minutes to form a single-cell suspension for FACS analysis. Lungs from 3 mice per group were instilled with formalin, fixed, embedded, and sectioned prior to H&E staining for histologic characterization of tumors and Ki67 IHC analyses.

Measurement of plasma treprostinil levels

Plasma was flash frozen and sent to Tandem Labs for determination of treprostinil levels. Samples were extracted with methanol, and an aliquot was injected onto an ultraperformance liquid chromatographic system equipped with a triple quadrupole tandem mass spectrometer (AB/MDS Sciei API-5000) detector operated in negative TurbolonSpray mode. Separation of treprostinil from extracted matrix materials was accomplished using a Water Acquity BEH C18 (2.1 × 100 mm, 1.7 μm) column operated at 65°C. Mobile phase A consisted of 0.1% formic acid in water and mobile phase B consisted of 0.1% formic acid in acetonitrile at a total flow rate of 0.775 mL/minute. Calibration standards of treprostinil diluted in control mouse plasma were used to construct standard curves. Linear-weighted (1/concentration2) regression analysis of peak area ratio versus theoretical concentration.
was used to produce calibration curves. These measurements were conducted according to published techniques (19).

**FACS analysis**

Single-cell suspensions from the collagenase lung digest were filtered twice through a 70-μm filter, and cells were harvested by centrifugation and counted. One million cells per sample were aliquoted into 96-well plates and blocked with FC Block (BD Pharmingen: 553142) prior to incubation with a fluorescently labeled lymphocyte panel of antibodies (CD3, PE, BD Pharmingen: 553064; CD4, APC, BD Pharmingen: 553051; and CD8, FITC, BD Pharmingen: 553031) or macrophage/neutrophil panel (MHCII, eFluor 450, eBioscience: 48-5321-82; Ly6G, PE, eBioscience: 12-6931-82; CD11b, PE-Cy7, eBioscience: 25-0114-82; CD11c, PerCP-Cy5.5, BD Pharmingen: 550993; and F4/80, APC, eBioscience: 17-4801-82). Fixed cells were then subjected to FACS analysis on a BD LSR II flow cytometer. Populations were determined using FlowJo software as described previously (20).

**CXCR4 expression**

RNA was extracted with a Qiagen RNeasy Kit from mouse lung tissue harvested from control, control/treprostinil, urethane, urethane/early treprostinil, and urethane/late treprostinil groups. Equal amounts of RNA were reverse transcribed with a Qiagen RT2 First Strand Kit. Samples were assessed for CXCR4 expression by qPCR using a validated Quantitect Primer Assay (Qiagen) and Quantitect SYBR Green qPCR Master Mix. Measurements were conducted in triplicate and were normalized to 60S ribosomal protein L13 (Bio-Rad Primer PCR Assay).

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**Figure 1.** Treprostinil MTD and tumor prevention study. Mice fed increasing doses of treprostinil in chow were weighed daily (A) for 3 weeks, and 28.8 mg/kg was determined to be the MTD. Treprostinil in chow (28.8 mg/kg chow) was administered 1 week before (ET) or 6 weeks after (LT) urethane (Ure) exposure, and tumor number (B), burden (C), and diameter (D) were measured in mice harvested 16 weeks after urethane exposure. Weight gain was similar between groups (E). Plasma treprostinil levels were measured in each group (F) at harvest, and consistent levels were found in each treprostinil-treated group.
Cell culture
JF32 cells were derived from a urethane-induced tumor in an A/J mouse (21) and cultured in MEM α media (Invitrogen: 12571-063) supplemented with 10% FBS (Sigma) and 1% penicillin (10,000 U/mL)/streptomycin (10,000 μg/mL)/ampicillin B (25 μg/mL) solution (Invitrogen: 30-004-CI). JF32 cells were tested for mycoplasma and authenticated by IDEXX RADIL in 2012. Cells used in this study were thawed from stocks frozen down from the tested cells and were passaged no more than 15 times after thawing. Cells were maintained in a humidified incubator at 37°C/5% CO2/95% ambient air, and split twice weekly. To measure proliferation, 2,000 cells per well were seeded into 24-well tissue culture plates and allowed to adhere overnight. Cells were serum starved for 24 hours prior to exposure to PGE2 (in DMSO, Cayman Chemicals: 14010), iloprost (in methyl acetate, Cayman Chemicals: 18215), or treprostinil (in DMSO, Cayman Chemicals: 10162) at the indicated concentrations for 48 hours. Cells were harvested and counted using a Celometer Auto T4 (Nexcelom).

Ki-67 proliferative index
Ki-67 immunostaining was conducted on tumors from treprostinil treated and control animals as described previously (22, 23). In brief, 5-μm lung sections from the urethane, ET, and LT groups were deparaffinized, peroxidases blocked with 3% hydrogen peroxide, and antigen retrieval performed in boiling Diva Decloaker (Biocare Medical, DV2004G1) reagent under pressure for 10 minutes. Sections were incubated with Ki67 primary Ab (1:25 dilution, Abcam 15580) for 30 minutes at room temperature, and Ki67+ nuclei were detected using Biocare Medical’s Mach 2 Detection Kit (MRCT523). Ki67-positive nuclei/mm2 were counted in each tumor and averaged within each group using an Olympus BX51 microscope fitted with an Olympus DP72 digital camera and CellSens Entry software (Olympus) to calculate tumor area.

Statistical analysis
Differences between two groups were analyzed by Student t test, and differences between three or more groups by one-way ANOVA followed by Student Newman–Keuls post hoc analysis (except where indicated). Results were considered significant at P < 0.05. EC50 values and statistical analyses were generated using GraphPad Prism (version 5.0, GraphPad Software, Inc.).

Results
MTD studies
The MTD for treprostinil dosing in chow was determined to be 28.8 mg/kg/day. Animals in the 75 mg/kg/day treprostinil chow group were sacrificed after the first week because of weight loss exceeding 15%. The mice in the remaining three groups (control, 14.4 and 28.8 mg/kg/day) chow gained equivalent weight during the remainder of the study (Fig. 1A). Treprostinil at these doses did not alter BAL levels of neutrophils, lymphocytes, and macrophages. PGE2 and PGI2 were unchanged with treprostinil exposure (Supplementary Fig. S1).

Treprostinil chemoprevention of urethane carcinogenesis
Mouse lung adenomas were induced by a single urethane exposure. To distinguish between prevention of initiation versus
prevention of tumor promotion, feeding of treprostinil chow (28.8 mg/kg/day) was initiated 1 week prior to carcinogen exposure (ET) or 6 weeks after (LT). Neither regimen affected urethane-induced tumor multiplicity, burden, or diameter (Fig. 1B–D). Histologic review of H&E slides from experimental and control animals revealed no discernable differences in histology between treatment groups (data not shown). Weight gain was similar between all treatment groups throughout the 20-week study (Fig. 1E). Tumor incidence for urethane-exposed mice in this study was 100%, with all urethane-exposed mice developing at least two tumors. There were no differences in any parameters measured between the ET and LT treprostinil groups. Plasma treprostinil levels (15–20 ng/mL) from control and urethane-treated mice fed treprostinil chow were similar regardless of treatment regimen (Fig. 1F). These levels are similar to those shown to be therapeutic for PAH in rat and human studies (19).

Inflammatory cell studies

To determine whether treprostinil treatment induced differences in lung tissue inflammatory cell populations, lung digests were examined by flow cytometry. Lymphocyte and macrophage/neutrophil marker panels were analyzed to quantify these populations in each group. Tissue levels of CD8$^+$ T lymphocytes were 5- to 6-fold higher in tumor-bearing treprostinil-treated mice compared with those of urethane-exposed AIN-76a-fed mice and control mice receiving either AIN76a or treprostinil chow (Fig. 2A and B). CD4$^+$ T lymphocyte numbers increased 2- to 3-fold in treprostinil-treated, tumor-bearing mice compared with those receiving urethane. Conversely, CD11b$^{hi}$CD11c$^{hi}$F4/80$^{hi}$ macrophage numbers decreased by approximately 50% in tumor-bearing mice fed treprostinil chow (Fig. 3A–C). No significant changes were observed in neutrophil (CD11b$^{hi}$, Ly6G$^+$) or CD11b$^{hi}$CD11c$^{mid}$F4/80$^{lo}$ interstitial/tissue macrophages (Fig. 3C).

CXCR4 expression

CXCR4 is a chemokine receptor whose expression has been shown to increase in response to treprostinil exposure in hematopoietic stem cells (24). CXCR4 message increased slightly, but insignificantly, in uninvolved tissue from treprostinil-treated, naïve mice and urethane-treated mice. Significant increases in CXCR4 message were detected in the LT group (Fig. 4), indicating that treprostinil was bioavailable and that these increases...
occurred in stromal tissue. Treprostinil did not increase CXCR4 expression in JF32 murine lung–derived cells (data not shown).

Effects of treprostinil exposure on tumor cell proliferation

To further examine the effects of treprostinil on adenocarcinoma, JF32 cells were exposed to increasing concentrations of PGE2, iloprost, and treprostinil for 48 hours in serum-free media. Cells were harvested and counted. Iloprost had no effect on cell proliferation at concentrations ranging from 0.3 nmol/L to 300 μmol/L (data not shown). PGE2 and treprostinil exposure increased JF32 cell numbers by 2.9- and 2.1-fold (respectively) over control media containing the DMSO vehicle (Fig. 5A and B), with EC50s of 750 nmol/L (PGE2) and 1.1 nmol/L (treprostinil). Although iloprost did not affect cell proliferation directly, JF32 cells pretreated with Iloprost did not exhibit PGE2 or treprostinil-induced increases in proliferation (Fig. 5C), indicating that these two prostacyclin analogues may have opposing functions in lung epithelial cells. Analysis of tumor cell proliferation in vivo using Ki67 staining (Fig. 5D) showed no significant differences in proliferation (Fig. 5E), indicating possible opposing effects of treprostinil activities in tumor and stromal cells. This supports our finding that tumor sizes were similar between all groups.

Discussion

Next-generation prostacyclin analogues like treprostinil have been developed to improve the ease of administration and dosing intervals that limit the utility of other prostacyclin analogues. Although oral iloprost improved bronchial dysplasia in our clinical trial, the oral formulation is currently not manufactured. Inhaled iloprost is currently being tested for clinical efficacy in former smokers with bronchial dysplasia, but the delivery options and stability of treprostinil could make it an alternative treatment option. We therefore tested whether orally delivered treprostinil exhibited lung chemopreventive properties similar to iloprost. In our studies, mice receiving treprostinil in two dosing regimens developed urethane-induced adenomas and adenocarcinomas that were equal in incidence (100%), multiplicity, and size to those in mice that received control Chow. In spite of its attractive pharmacokinetic profile and available formulations, our preclinical testing demonstrated that treprostinil does not share iloprost’s chemopreventive properties. As the levels achieved in plasma were similar to those shown to be therapeutic in rats and humans (19), and treprostinil exposure altered lung inflammatory cell numbers (specifically increasing CD4+ and CD8+ T lymphocytes and decreasing CD11b+ macrophages in tumor-bearing animals) and increased CXCR4 expression in stromal tissue, we conclude that the lack of treprostinil efficacy was not due to poor bioavailability.

Iloprost and treprostinil are both prostacyclin analogues, share structural similarities, and both bind and activate the single prostacyclin receptor (IP). However, similar to many prostaglandin analogues, each compound activates a distinct set of related receptors at similar physiologic concentrations. Iloprost binds prostaglandin E2 receptor 1 (EP1; 0.3 nmol/L) and IP (0.4 nmol/L) but requires micromolar concentrations to activate EP2 (2.1 μmol/L) and DP1 (2.1 μmol/L). In contrast, treprostinil activates DP1 (0.6 nmol/L), IP (1.9 nmol/L), and EP2 (6.2 nmol/L) at similar concentrations, but activates EP1 at much higher levels (290 nmol/L; ref. 8). Our plasma treprostinil levels reached 15 to 20 ng/mL in all of our treatment regimens, indicating that EP1 receptor was not activated. Optimal dosing for chemoprevention studies has not been determined, and there are studies suggesting that cyclical dosing (as opposed to the continuous dosing required for the treatment of PAH) of prostacyclin analogues is more effective in inhibiting cancer metastasis (25). To our knowledge, this is the first study to examine treprostinil-impregnated Chow in murine lung cancer experiments.

Our group has previously shown that EP2-null mice develop fewer urethane-induced lung tumors than their wild-type littermates (26), so the fact that treprostinil activates the EP2 receptor may explain its lack of chemopreventive efficacy in this preclinical model. Iloprost activates PPARγ independent of the IP receptor (27), but treprostinil requires the IP receptor to activate PPARγ and even in its presence may have little effect on PPARγ activity (28, 29). PPARγ activation has been shown to prevent lung tumors in a variety of studies (30, 31). In a study of the effects of prostacyclin analogues on macrophage function, treprostinil increased phagocytosis blocks, bacterial killing, and cytokine generation, activities more closely resembling those of the pro-tumorigenic PGE2 than the antitumorigenic PG12 (32). One or more of these differential activities could account for the reduced chemopreventive efficacy of treprostinil.

Another potential explanation for differences between the two agents may be related to their differential activation of chemokine receptor type 4 (CXCR4). CXCR4 is an α-chemokine receptor with potent chemotactic activity for lymphocytes (33) that is known to be involved in therapeutic resistance and epithelial-to-mesenchymal transition in NSCLC. Elevated CXCR4 expression correlates with aggressive metastasis, advanced stage disease, and shorter overall NSCLC survival rates (34). A recent publication by Kazemi and colleagues demonstrated that treprostinil (and not iloprost or beraprost) increased CXCR4 expression in hematopoietic stem and progenitor cells (HSPC), enhanced CXCR4-mediated HSPC migration, and improved the engraftment of HSPCs in lethally irradiated mice without impairing their self-renewal capacity (24). The enhanced ability of treprostinil to boost CXCR4-related...
Trentrostenil Does Not Prevent Murine Lung Cancer

Figure 5.
PGE2 and treprostinil increase proliferation of mouse lung adenocarcinoma cells in vitro but not in vivo. JF32 mouse lung adenocarcinoma cells were serum-starved for 24 hours prior to incubation with increasing doses of PGE2 (A) or treprostinil (B) in serum-free media. Cells were harvested by trypsinization and counted. **P < 0.01 versus control. ***P < 0.001 versus control. Graphs represent two independent experiments with at least three samples/group in each experiment. D and E, Ki67** staining (D) was used to generate a proliferative index (E). Ki67 nuclear staining (red arrowhead) was counted in each tumor. Area of each tumor was determined using CellSens Entry software, and the number of Ki67** nuclei/mm$$^2$$ tumor was determined in each group. No differences were detected between treatment groups.

pathway activity may explain its lack of chemopreventive properties.

In PGIS-overexpressing mice, PGIS transgene expression is controlled by the surfactant protein C promoter so that these mice produce excess PGI$$\text{3}$$ only in lung type II and club cells (35). This overproduction protects mice from developing lung tumors. These mice also show increased BAL macrophages numbers. Iloprost exposure in chow results in similar reduction in tumor number and increase in alveolar macrophages (9). Alveolar macrophage numbers in treprostinil-exposed mice were comparable with naive mice and were significantly decreased in treprostini-exposed tumor-bearing mice. Treprostinil’s lack of chemopreventive efficacy could be a result of direct effects on stromal cells. Prostaglandin analogs (including iloprost and treprostinil) have been shown to suppress lipopolysaccharide (LPS)-induced TNF$$\alpha$$ expression in monocyte-derived dendritic cells (38). Yeh and colleagues proved these effects were IP receptor-mediated. Iloprost exposure in serum-free media did not reverse the suppression. Iloprost also regulated the expression of costimulatory molecules CD40, HLA-DR, CD80, and CD86 (36). An earlier study of PGJ$$\text{2}$$ analogues and proinflammatory cytokines showed that iloprost and cicaprost, but not treprostinil, suppressed LPS-induced expression of CD86, CD40, and MHC class II molecules by bone marrow–derived dendritic cells (BMDCs) and inhibited the ability of BMDCs to stimulate antigen-specific CD4$$\text{+}$$ T-cell proliferation and production of IL5 and IL13 (37). In addition, iloprost and treprostinil exposure differentially affected murine lung adenocarcinoma cell proliferation. Treprostinil promoted cell growth at a magnitude similar to that of PGE$$\text{2}$$ at a much lower concentration, and this effect was abrogated by iloprost pretreatment. Identifying and comparing iloprost and treprostinil receptor-binding activity in epithelial, dendritic, and stromal cells could elucidate the mechanism of iloprost chemoprevention and guide us toward a more effective chemopreventive prostacyclin-based agent in the future.

Disclosure of Potential Conflicts of Interest

R.L. Keith has ownership interest (including patents) in uses of prostacyclin analogs for cancer chemoprevention and patent (preliminary application: Identifying biomarkers to predict prostacyclin analog response for the chemoprevention of cancer and reversal of pre-malignancy) and is a scientific/ advisory board member for LLN-Gevity Foundation. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


Grant Support

Each author’s financial support, including the recipient name (e.g., J.A. Smith), funder name, and associated grant number(s) are as follows: Lori D Dwyer-Nield–Skaggs School of Pharmacy and Pharmaceutical Sciences ADR Seed Grant (L. Dwyer-Nield principal investigator), NCIPS0

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