

RAPID REPORT

AMD3100 ameliorates cigarette smoke-induced emphysema-like manifestations in mice

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Barwinska D, Oueini H, Poirier C, Albrecht ME, Bogatcheva NV, Justice MJ, Saliba J, Schweitzer KS, Broxmeyer HE, March KL, Petrache I. AMD3100 ameliorates cigarette smoke-induced emphysema-like manifestations in mice. *Am J Physiol Lung Cell Mol Physiol* 315: L382–L386, 2018. First published May 10, 2018; doi: 10.1152/ajplung.00185.2018.—We have shown that cigarette smoke (CS)-induced pulmonary emphysema-like manifestations are preceded by marked suppression of the number and function of bone marrow hematopoietic progenitor cells (HPCs). To investigate whether a limited availability of HPCs may contribute to CS-induced lung injury, we used a Food and Drug Administration-approved antagonist of the interactions of stromal cell-derived factor 1 (SDF-1) with its chemokine receptor CXCR4 to promote intermittent HPC mobilization and tested its ability to limit emphysema-like injury following chronic CS. We administered AMD3100 (5mg/kg) to mice during a chronic CS exposure protocol of up to 24 wk. AMD3100 treatment did not affect either lung SDF-1 levels, which were reduced by CS, or lung inflammatory cell counts. However, AMD3100 markedly improved CS-induced bone marrow HPC suppression and significantly ameliorated emphysema-like end points, such as alveolar airspace size, lung volumes, and lung static compliance. These results suggest that antagonism of SDF-1 binding to CXCR4 is associated with protection of both bone marrow and lungs during chronic CS exposure, thus encouraging future studies of potential therapeutic benefit of AMD3100 in emphysema.

bone marrow; COPD; emphysema; lung treatment

INTRODUCTION

Pulmonary emphysema, a major manifestation of chronic obstructive pulmonary disease (COPD), is characterized by

loss of alveolar units, typically as a result of prolonged exposure to cigarette smoke (CS). We have shown that CS reduces the number and function of hematopoietic progenitor cells (HPCs) in the bone marrow (BM) of mice (26). It is increasingly apparent that BM-derived cells are involved in tissue repair, including that of injured lungs (1, 23, 24, 28). Furthermore, numbers of circulating HPCs are inversely correlated with the severity of COPD (9, 12, 17). The chemokine receptor CXCR4 and its main ligand, stromal cell derived factor-1 (SDF-1/CXCL12), have been implicated in migration of various stem/progenitor cell types (3, 4, 5, 6) and in COPD (8, 14, 20). We therefore hypothesized that the reduction of HPC is directly linked to the lung's inability to repair CS-induced damage. To test whether increased mobilization of HPCs from the BM will reduce CS-induced lung injury, we utilized a Food and Drug Administration-approved drug (18), AMD3100, that reversibly blocks the CXCR4 receptor's interaction with CXCL12 without influencing CXCR4 expression required for the retention of HPC within the BM (5, 6). Using a mouse model of CS-induced chronic lung injury, we show that AMD3100 protects against airspace enlargement and lung dysfunction.

MATERIALS AND METHODS

Reagents and materials. All reagents, including AMD3100, were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise specified. 3R4F reference cigarettes were from Tobacco Research Institute (Lexington, KY).

Animals. Animal studies were only performed at Indiana University School of Medicine and were approved by the Indiana University Animal Care and Use Committee. C57Bl/6 mice ($n = 5$ /group, females, 8–10 wk old, Jackson Laboratories, Bar Harbor, ME) were exposed 5 h/day to 11% mainstream and 89% sidestream smoke using a Teague 10E whole body exposure apparatus (Teague Enterprises, Woodland, CA) for the indicated time (21). A group of mice exposed to ambient air only were used as controls. Given that AMD3100 has been extensively studied in preclinical and clinical

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studies (10), we did not include a drug-only group in this proof of principle study. CS-exposed mice received daily subcutaneous injections of AMD3100 ($5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$) or vehicle (PBS) for 5 consecutive days per cycle, during weeks 1, 12, and 22 (Fig. 1A) of CS exposure. The first dose of the drug was administered at the beginning of the initial CS exposure, concomitantly with CS exposure. The rationale for this design is based on previous reports (16) that indicated that the effects of daily AMD3100 were rapid but become undetectable following the seventh dose.

Clonogenic progenitor cell assay. The number of HPCs, consisting of granulocyte-macrophage colony-forming units, erythroid burst-forming units, and multipotential granulocyte, erythroid, monocyte, megakaryocyte colony-forming units, was determined as described in Ref. 21. HPCs were collected by flushing the left and right femurs with DMEM and plated at a density of 4.5×10^4 cells per 35 mm dish in 0.9% methylcellulose culture medium with supplements and growth factors using Hemavet 950FS (Drew Scientific, Dallas, TX) (21).

Lung function testing and lung histology. Mice were anesthetized with ketamine-isoflurane, intubated via trachea, and mechanically ventilated (140 breaths/min, tidal volume 0.3 ml, positive end-expiratory pressure 5 cmH_2O) to measure lung function with FlexiVent (Scireq, Montreal, Canada). Following euthanasia, lungs were inflated with 0.25% (vol/vol) agarose in 10% (vol/vol) formalin-PBS at a constant pressure of 20 cmH_2O . Paraffin-embedded lung

tissue sections were hematoxylin-eosin- or trichrome Masson-stained, and mean linear intercepts (MLI) and surface to volume ratios were determined using unbiased automated morphometry, as described (13).

Bronchoalveolar lavage fluid assessment. Bronchoalveolar lavage (BAL) fluid was obtained as described in Ref. 21. SDF-1 was measured in the BAL fluid using Mouse CXCL12/SDF-1 alpha Quantikine ELISA Kit (R&D Systems, Minneapolis, MN). Leukocyte counts and differentials were determined by preparing cytopins of BAL fluid using Shandon CytoSpin III (Thermo Fisher Scientific, Waltham, MA) at 750 g for 3 min. Cytopin slides were then fixed and stained using Richard-Allan Scientific Three-Step Stain (Thermo Fisher Scientific) using fixative, solution A, and solution B for 15 s each. For the differential, 250 cells were classified per slide.

Statistical analyses. Statistical analyses using GraphPad Software (La Jolla, CA) were performed using one-way ANOVA with Dunnett's post hoc analysis for intergroup comparisons; $P < 0.05$ was considered statistically significant.

RESULTS

Consistent with our previous report (26), we observed early depletion of HPC in the BM of mice exposed to CS (Fig. 1B). To ascertain that AMD3100 does not deplete the BM pool of

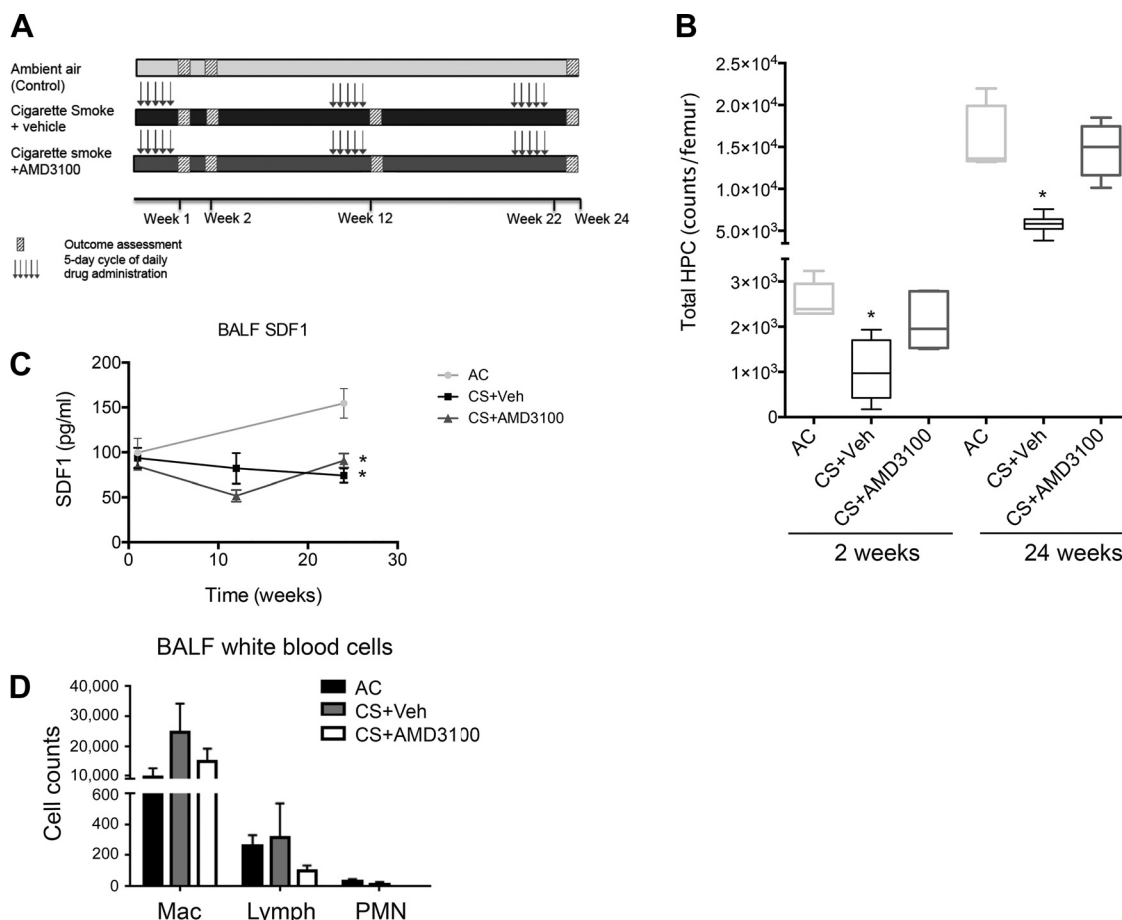


Fig. 1. Impact of AMD3100 on bone marrow (BM) progenitor cells and lung inflammatory cells during cigarette smoke (CS) exposure. **A**: schematic with experimental design; AMD3100 ($5 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$, SQ) or vehicle (Veh) control was administered in cycles of 5 consecutive daily injections. Striped boxes indicate time of tissue harvesting for analyses. **B**: total numbers of hematopoietic progenitor cells (HPCs) in BM of mice. Horizontal line shows median. **C**: stromal derived factor-1 (SDF-1) levels measured in bronchoalveolar lavage fluid (BALF). Horizontal line shows median. **D**: total numbers of inflammatory cells recovered in BALF. $n = 5/\text{group}$. Means \pm SE, $P < 0.05$ by one-way ANOVA, $*P < 0.05$ measured with Dunnett's post hoc multiple comparisons test. PMN, polymorphonuclear cells (neutrophils); AC, ambient air control exposure.

HPCs, we assessed the number of HPCs in BM of CS-exposed mice receiving a 5-day cycle of AMD3100 administered daily. We could not detect HPC depletion, but instead, mice treated with AMD3100 exhibited a rather marked restoration of BM HPC after 2 wk of CS exposure following a single 5-day cycle of drug treatment (Fig. 1B). Similarly, after three 5-day cycles of AMD3100 treatment administered intermittently during a chronic 6-mo CS exposure, the total HPCs (Fig. 1B) that encompassed subpopulations of granulocyte-macrophage colony-forming units, erythroid burst-forming units, and multipotential granulocyte, erythroid, monocyte, megakaryocyte colony-forming units in the BM were significantly higher compared with vehicle-treated animals. AMD3100 administration did not affect

lung SDF-1 levels measured in the lung BAL fluid, which were significantly decreased by chronic CS exposure (Fig. 1C). There were also no significant changes in the number of total white blood cells or inflammatory cell subsets recovered in the BAL fluid of animals exposed to CS and treated with AMD3100 (Fig. 1D). However, AMD3100 treatment significantly ameliorated CS-induced increases in airspace size, assessed by hematoxylin-eosin staining of lung parenchyma (Fig. 2A) and measured by mean linear intercepts and surface to volume ratios determined by automated morphometry in a blinded fashion (Fig. 2, B and C). Furthermore, AMD3100-treated animals had significant improvement in lung static compliance compared with vehicle-treated CS-exposed animals (Fig. 2D). The effect of

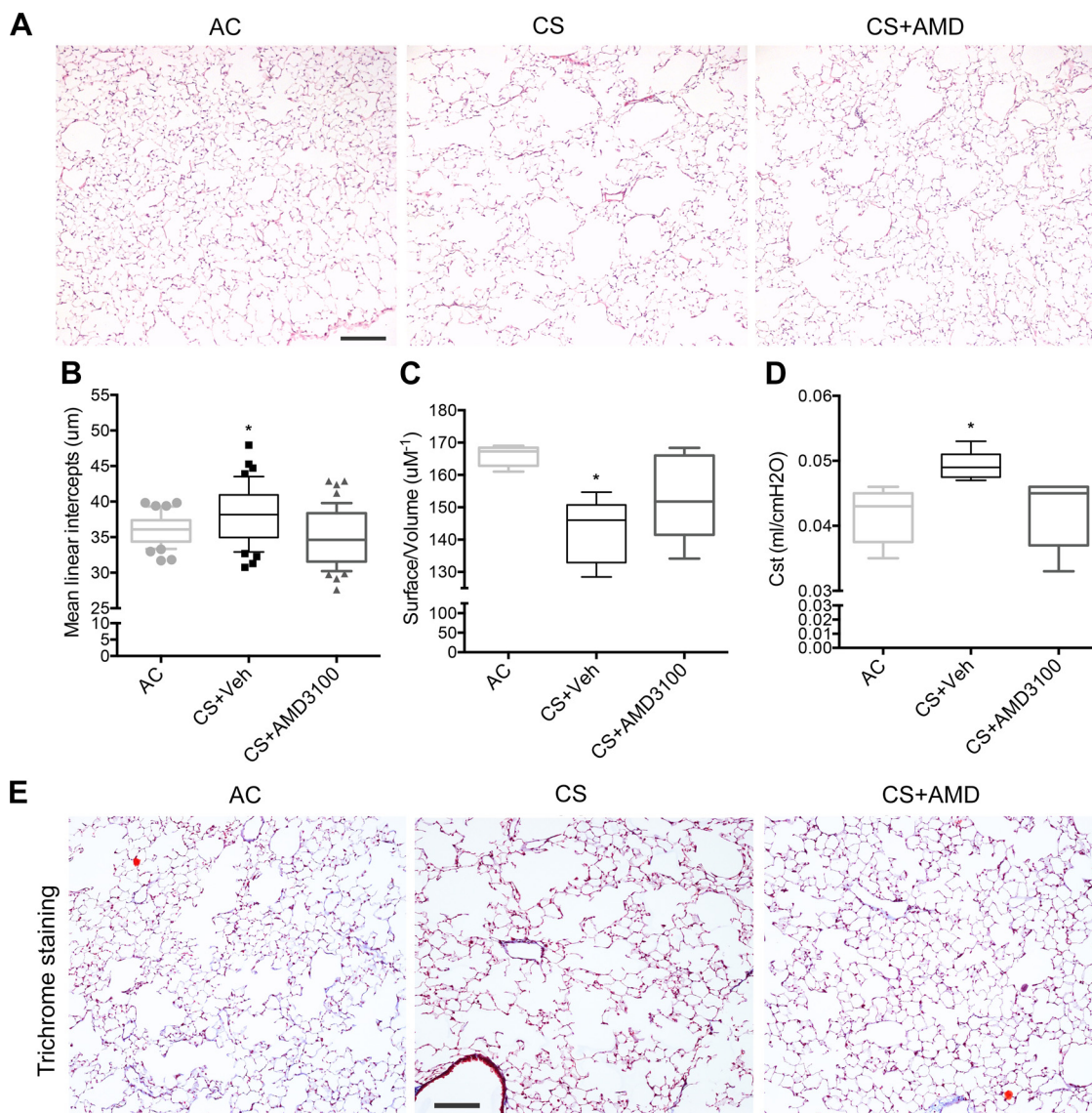


Fig. 2. Effect of AMD3100 on airspace size and fibrosis in mouse lung parenchyma during chronic cigarette smoke (CS) exposure. *A*: representative micrographs ($n = 5$) of parenchyma of hematoxylin-eosin stained lungs following inflation at constant pressure (size bar: 100 μm). *B*: mean linear intercepts of mouse lung parenchyma determined by automated morphometry. *C*: surface/volume ratios of mouse lung parenchyma determined by automated morphometry. *D*: static lung compliance of mice. *E*: representative ($n = 3$) lung parenchyma images showing trichrome-stained interstitium (size bar: 100 μm). Note lack of appreciable lung fibrosis in any of the groups. All box plots showing 10th and 90th percentile of $n = 5$; $P < 0.05$ by one-way ANOVA; * $P < 0.05$ measured with Dunnett's post-hoc multiple comparisons test. Veh, vehicle; Cst, static lung compliance; AC, ambient air control exposure.

AMD3100 on lung compliance cannot be explained by fibrotic stiffening of the lung since the drug did not increase lung collagen content, as assessed by trichrome Masson staining (Fig. 2E).

DISCUSSION

Our results indicate that intermittent AMD3100 administration attenuates CS-induced emphysema-like morphological and functional changes in mouse lungs associated with preservation of HPC levels in the BM. Our report builds on our recent finding that even brief CS exposures reduce HPC populations in the BM (26) and the notion that HPC recruitment to the lung contributes to its protection against chronic CS exposure. Since, to our knowledge, the effects of sustained HPC mobilization from the BM have not been reported, we designed our experiments in mice using a regimen of a single pulsed (*week 1*) or intermittent (*weeks 1, 12, and 22*) pulses of AMD3100 treatment. These approaches did not cause HPC depletion following AMD3100 injection. This may be explained by the fact that the pharmacological induction of HPC mobilization to the peripheral blood is short-lived, occurring within minutes to 2 h, and is reversible (5). In addition, we observed a protective effect of AMD3100 on the number of HPCs in the BM even following chronic CS exposures. While the mechanisms for this effect remain to be demonstrated, it is possible that mobilized cells, which acquire superior homing properties (3), may return to their niche and repopulate the BM. Since the mobilized HPCs are predominantly in G₀ (5), it is unlikely that an increase in their proliferation rates while in peripheral blood would explain their increased BM abundance following AMD3100, although we cannot rule out that increased proliferation precedes their mobilization. While AMD3100 has been used and approved for clinical use alone, AMD-3100 plus the cytokine granulocyte colony stimulating factor (G-CSF) have been shown to synergize with AMD3100 to enhance the mobilization of HPCs (5, 15). Since G-CSF works through a mechanism other than CXCR4 to mobilize HPCs, we focused on AMD3100 alone in our studies. However, in the future, it may be of interest to evaluate the combined effects of G-CSF plus AMD3100 in these studies.

The protective effects of AMD3100 on CS-induced chronic lung injury raise the possibility that BM mobilization increases the availability of HPCs for lung cell maintenance or repair, particularly necessary since both the number and proliferative potential of BM HPCs are reduced upon CS exposure (21, 26). Quantitative analyses of HPCs in the BM of animals treated with AMD3100 support the notion that the beneficial effects of the drug are related to increased HPCs available for lung repair. However, HPC changes may have been difficult to detect at 10 days after AMD3100 administration when we assessed CS-induced lung injury since previous studies documented a peak of HPC mobilization into blood at 1 h post-treatment (5, 22). There may be multiple reasons for the lack of curative effects of different types of progenitor cell therapy for COPD in clinical trials, many of which have been recently reviewed (25). Although patient selection, type of cells, and disease phenotype may have had a marked effect on outcomes, the results in human subjects may challenge the notion that the effects of HPCs in mice can be translated to humans or that BM HPCs directly contributed to beneficial effects noted in our

model. Indeed, the mobilization of BM HPCs may not be the exclusive mechanism mediating the lung protective effects of AMD3100. SDF-1-CXCR4 signaling on resident lung cells may also be involved in tissue responses to CS-induced injury, independent of HPC mobilization and lung recruitment. Furthermore, lung recruitment of macrophages and neutrophils with critical roles in emphysema pathogenesis can be altered by CXCR4 inhibitors, such as AMD3100 (7) or 4F-benzoyl-TE14011 (27). In our model, however, we did not identify differences in leukocyte numbers following AMD3100. HPCs may also be recruited to the lung via local SDF-1 production, which was decreased by CS. Although AMD3100 did not affect SDF-1 levels in the lung, transient increases in SDF-1, which have been described within 24 h of AMD3100 administration and became negligible at *day 7* (16), cannot be ruled out. However, since the drug would block SDF-1 interaction with CXCR4 on HPCs, it is unlikely that the effects seen were due to increased ability of the lung to attract HPCs. Although the interactions of CXCR4 with other ligands, including MIF and ubiquitin (2, 19), may also be considered, there is no evidence that AMD3100 has any effect on the expression levels of CXCR4, and therefore these have not been measured in our study.

In conclusion, together with reports of decreased blood levels and increased sputum levels of CXCR4-positive HPCs in COPD patients (20) and altered SDF-1 and/or CXCR4 levels in COPD (8, 14), our results support increasing interest in the interaction between SDF-1-CXCR4 as a target for lung repair in COPD. Future investigations of AMD3100 effectiveness when administered after the onset of emphysema, which was not assessed in this study, would be informative in future preclinical testing, along with a more detailed elucidation of the mechanisms by which CXCR4 signaling within and outside the context of SDF-1 interactivity contributes to lung homeostasis or airspace enlargement. Although the long-term administration of AMD3100 is not devoid of side effects (6, 11), our report supports usefulness of this Food and Drug Administration-approved drug as a potential treatment for emphysema.

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DISCLAIMERS

The content of this article does not represent the views of the US Department of Veteran Affairs or the United States Government.

DISCLOSURES

A provisional patent for COPD treatment with AMD3100 was filed by Drs. Petrache, Oueini, and March.

AUTHOR CONTRIBUTIONS

H.E.B., K.L.M., and I.P. conceived and designed research; D.B., H.O., C.P., M.E.A., M.J.J., J.S., and K.S.S., performed experiments; D.B., H.O., C.P.,

N.V.B., and I.P. analyzed data; D.B., H.O., C.P., N.V.B., and I.P. interpreted results of experiments; D.B., H.O., and I.P. prepared figures; D.B., H.O., N.V.B., H.E.B., and I.P. drafted manuscript; H.E.B. and I.P. edited and revised manuscript; H.E.B., K.L.M., and I.P. approved final version of manuscript.

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