ARTICLE IN PRESS

Life Sciences xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

5-HT$_2$ receptor activation alleviates airway inflammation and structural remodeling in a chronic mouse asthma model

Thomas W. Flanagan[a], Melaine N. Sebastian[a], Diana M. Battaglia[b], Timothy P. Foster[b], Stephania A. Cormier[c], Charles D. Nichols[a,*]

[a] Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, New Orleans, LA, 70112, USA
[b] Department of Microbiology, Immunology, And Parasitology, Louisiana State University Health Sciences Center, New Orleans, LA, 70112, USA
[c] Department of Biological Sciences, Louisiana State University, 202 Life Sciences Building, Baton Rouge, LA, 70803, USA

ARTICLE INFO

Keywords:
Serotonin
Asthma
5-HT
5-HT2A agonists
5-HT signaling
Inflammation
Psychedelic
Fibrosis

ABSTRACT

Aims: Although the bulk of research into the biology of serotonin 5-HT$_{2A}$ receptors has focused on its role in the CNS, selective activation of these receptors in peripheral tissues can produce profound anti-inflammatory effects. We previously demonstrated that the small molecule 5-HT$_2$ receptor agonist (*R*)-2,5-dimethoxy-4-iodoamphetamine [(*R*)-DOI] inhibits TNF-α-mediated proinflammatory signaling cascades and inflammation via 5-HT$_{2A}$ receptor activation and prevents the development of, and inflammation associated with, acute allergic asthma in a mouse ovalbumin (OVA) model. Here, we investigated the ability of (*R*)-DOI to reverse inflammation and symptoms associated with established asthma in a newly developed model of chronic asthma.

Methods: An 18-week ovalbumin challenge period was performed to generate persistent, chronic asthma in BALB/c mice. Four once daily intranasal treatments of (*R*)-DOI were administered one week after allergen cessation, with respiratory parameters being measured by whole-body plethysmography (WBP). Cytokine and chemokine levels were measured by quantitative real-time polymerase chain reaction (qRT-PCR) in homogenized lung tissue, bronchoalveolar (BALF) fluid was analyzed for chemokine modulation by multiplex assays, and Periodic Acid-Schiff and Masson's Trichrome staining was performed to determine goblet cell infiltration and overall changes to lung morphology.

Key findings: 5-HT$_2$ activation via (*R*)-DOI attenuates elevated airway hyperresponsiveness to methacholine, reduces pulmonary inflammation and mucus production, and reduces airway structural remodeling and collagen deposition by nearly 70%.

Significance: Overall, these data provide support for the therapeutic potential of (*R*)-DOI and 5-HT$_2$ receptor activation for the treatment of asthma, and identifies (*R*)-DOI as a novel therapeutic compound against pulmonary fibrosis.

1. Introduction

Of the 14 distinct serotonin receptor subtypes found in mammals, each of which is encoded by a specific gene [1], the 5-HT$_{2A}$ receptor is the most widely expressed throughout the brain and body [2–4]. The highest expression levels are found in the brain in areas like the cortex and claustrum, which mediate higher order processes such as memory and cognition [5–7]. Research into the function of this receptor has primarily focused on its role in the brain, and its role as the primary target of classic serotonergic hallucinogenic drugs, or psychedelics, in mediating their behaviors [8,9]. In peripheral tissues, the 5-HT$_{2A}$ receptor has been detected in nearly all cell types and tissues examined including epithelial and endothelial cells [10–12], renal cells [13], lymphocytes [14], fibroblasts [15], and hepatic cells [16], where it is implicated in cellular differentiation and proliferation. Interestingly, compared to other serotonin receptor subtypes, 5-HT$_{2A}$ receptor mRNA is expressed at elevated levels in several immune related tissues such as the spleen, thymus, and circulating lymphocytes [17].

5-HT$_{2A}$ receptors are also functionally expressed in a number of components that underlie the pathophysiology of asthma and the inflammation associated with asthma (CD4$^+$ T cells, alveolar macrophages, eosinophils, and lung epithelial and bronchial smooth muscle)

* Corresponding author. Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, 1901 Perdido St, New Orleans, LA, 70112, USA.
E-mail address: cnich1@lsuhsc.edu (C.D. Nichols).

https://doi.org/10.1016/j.lfs.2019.116790
Received 16 May 2019; Received in revised form 9 August 2019; Accepted 24 August 2019
0024-3205/ © 2019 Published by Elsevier Inc.

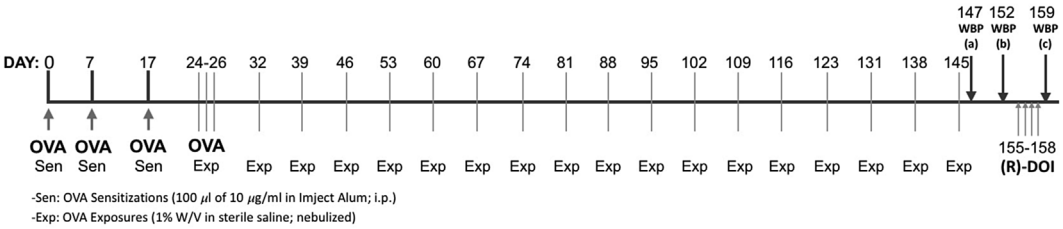

Fig. 1. Sensitization and challenge protocol for induction of chronic OVA-induced asthma in mice. Six-week-old male BALB/c mice received three intraperitoneal sensitizations with 20 μg chicken egg ovalbumin emulsified in 2 ml Imject Alum (100 μl) on days 0, 7, and 17. From days 24 to 26 mice were challenged with either 1% OVA in sterile saline or sterile saline aerosol for 20 min, followed by once weekly challenges for 19 weeks. On days 147 and 152 PenH was measured to validate the generation and persistence of hyperresponsiveness to methacholine. On days 155 to 158 an ultrasonic nebulizer was utilized to administer 1.0 mg/kg of (*R*)-DOI or vehicle control to treatment and control groups nose only. PenH was measured again measured on day 158 followed by humane euthanasia on day 160 to isolate serum, lungs, and BALF.

[17]. For example, 5-HT_{2A} receptor activation is crucial for eosinophil migration [18], and 5-HT_{2A} receptors have been shown to be involved in bronchial hyperresponsiveness [19]. Asthma itself is broadly defined as a chronic inflammatory disorder of the airways characterized by airflow obstruction, mucus hyperproduction, bronchial hyperresponsiveness, and airway inflammation that ultimately leads to airway structural remodeling and difficulty with breathing [20,21]. The pathophysiology of the disease stems from the increased expression of a variety of proinflammatory mediators from cells such as eosinophils, mast cells, activated T helper lymphocytes, and macrophages, which form a positive feedback loop to induce the recruitment of additional inflammatory cells and promote the symptoms of asthma [22–27]. We have previously discovered that 5-HT_2 receptor activation with psychedelics can induce potent anti-inflammatory responses [28]. For example, the 5-HT_2 receptor selective agonist and psychedelic (*R*)-2,5-dimethoxy-4-iodoamphetamine (*R*)-DOI potently blocks TNF-α-mediated inflammation both *in vitro* and *in vivo* at sub-behavioral levels [29,30]. We subsequently demonstrated that (*R*)-DOI potently suppresses the development of allergic asthma and associated pulmonary inflammation in the traditional ovalbumin (OVA) mouse model of acute allergic asthma [31]. In this model, nebulized nasal administration of (*R*)-DOI at doses as low as 0.01 mg/kg prior to OVA challenge fully prevents mucus overproduction, airways hyperresponsiveness (AHR) to methacholine (MeCh), eosinophilia, and pulmonary inflammation [6]. Of note, (*R*)-DOI-treatment prevented the expression of some, but not all, inflammatory markers assayed. For example, cytokines IL-5, IL-13, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were all significantly decreased, whereas IL-4 expression remained unaffected. These data suggest that unlike steroidal medications that have broad immunosuppressive activity, (*R*)-DOI has limited effects on the overall immune response, but sufficient targeted inhibition of inflammatory mechanisms to prevent asthma pathobiology. Therefore, we have proposed that (*R*)-DOI and related compounds represent a novel attractive steroid sparing small molecule based therapeutic strategy to treat asthma.

To extend our understanding of the therapeutic potential (*R*)-DOI has as an anti-inflammatory agent to treat asthma in the clinic, we examined the effects of (*R*)-DOI to alleviate and reverse *pre-existing symptoms* of asthma. To test the effects of (*R*)-DOI on persistent asthma we developed a new model of allergic asthma where relevant symptoms persisted for at least two weeks following the final allergen exposure, including robust structural remodeling of the airways. We found that consecutively challenging mice with aerosolized OVA for a period of 18 weeks, delivered once weekly, after the initial sensitization produced robust asthma-like symptoms and airway remodeling that persisted without decline for at least 14 days. Treatment with inhaled (*R*)-DOI 10 days after the final OVA challenge resulted in significantly reduced pulmonary inflammation, reversed collagen deposition, and improved airway function. Interestingly, many of the cytokines that we previously identified as being down regulated in the lung by (*R*)-DOI treatment in the acute OVA model were not reduced in this model by (*R*)-DOI. Overall this data provides new evidence for the therapeutic potential of (*R*)-DOI and 5-HT_2 receptor activation for the treatment of asthma.

2. Materials and methods

2.1. Drugs and reagents

(*R*)-DOI (provided by Dr. David E. Nichols at Purdue University, West Lafayette, IN) was dissolved in sterile saline prior to each use. Ovalbumin and methacholine were purchased from Sigma-Aldrich (St. Louis, MO).

2.2. Animals

For inhalation/asthma experiments, six-week-old male pathogen free BALB/c mice were purchased from Envigo (Somerset, NJ). All mice were maintained in the animal care facility at the Louisiana State University Health Sciences Center (New Orleans, LA) in ventilated cages in a pathogen-free environment with *ad libitum* access to food and water in a 12-h L/D cycle. All protocols were in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center.

2.3. OVA-induced chronic allergic airway inflammation

Sensitization-challenge protocols used in this study are summarized in Fig. 1, and are a modification from an existing protocol [33]. Essentially, mice were sensitized with an intraperitoneal injection (100 μl) of 20 μg Grade V chicken egg OVA (Sigma-Aldrich, St. Louis, MO) emulsified in 2 ml Imject Alum [$Al(OH)_3/Mg(OH)_2$; Pierce, Rockford, IL] on days 0, 7, and 17. Mice were subsequently challenged with an OVA aerosol [1% (wt/vol) OVA solution in sterile saline (Baxter Healthcare Corp., Deerfield, IL) generated using a VixOne reusable nebulizer (Westmed, Tucson, AZ) driven by a PARI PRONEB Ultra compressor (Pari Proneb, Midlothian, WA)] for 20 min on days 24–26, followed by once weekly challenges for an additional 17 weeks. Naive mice were challenged with a nebulized saline solution on the same schedule. On days 153–157, ten days after the final OVA exposure, each mouse was treated with 1.0 mg/kg (*R*)-DOI (nose-only inhalation) or vehicle control once daily using an ultrasonic nebulizer (Aeroneb Pro, Aerogen, Galway, Ireland).

2.4. Non-invasive determination of airway function

Airway function was measured in unrestrained, conscious mice using a single chamber, whole-body plethysmography (WBP) system (Buxco Electronics, Troy, NY, and EMKA Technologies, Falls Church, VA). Enhanced pause (PenH) was used as an index of airway

responsiveness as previously described [34]. Essentially, the animals were placed in a single chamber for an acclimatization period of 10 min followed by measuring the baseline PenH for 3 min prior to challenge. After 1 min exposures to the aerosolized bronchoconstrictor methacholine (MeCh, Sigma-Aldrich) at increasing concentrations (0, 3.125, 6.25, 12.5, and 50 mg/ml in isotonic saline) PenH was measured again for 3 min. Maximum PenH was expressed as the average maximal fold increase for each concentration of MeCh compared with PenH values after saline challenge and plotted as percent change from vehicle controls. n = 6 mice per treatment group.

2.5. Lung histopathology

Lungs were isolated and prepared as previously described [35]. Briefly, 12 h after the final WBP measurement, lungs were removed and inflated by gentle infusion of 10 ml Zinc Formal-Fixx Concentrate (Thermo Scientific, Shandon, Inc., Pittsburgh, PA) [36]. Fixed lungs were dehydrated in an ascending percentage of ethanol, embedded in paraffin, and sectioned at 4 µm. Each lung section was stained with periodic acid-Schiff to visualize mucus and imaged as previously described [35]. Adjacent sections were stained with Masson's trichrome to visualize collagen deposition. Morphometric analysis of Masson's trichrome-positive peribronchiolar collagen staining have been described elsewhere [37]. Briefly, colored images were processed using ImageJ (Wyne Rasband, National Institutes of Health, Bethesda, MD) to produce maps showing collagen stained areas within a bronchiole. The area of Masson's trichrome staining was normalized by the average of three different measurements of the diameter of the same bronchiole to reduce the influence of bronchiole caliber on the extent of collagen deposition. Results are expressed as the mean of the area of Masson's trichrome staining per micrometer length of basement membrane of brionchioles (roughly 150–200 µm internal diameter). Data are representative of at least 20 bronchioles of 5 animals per treatment group scored by observers blinded to the treatment conditions.

2.6. Cytokine and chemokine analysis by qRT-PCR

Expression levels of cytokine and chemokine mRNAs were determined using reverse transcription and quantitative real-time PCR (qRT-PCR) from lungs harvested 12 h following final WBP. RNA was extracted with TRIzol reagent (Life Technologies, Carlsbad, CA) for all lung tissues following the manufacturer's instructions. RNA was processed into first-strand cDNA using the ImProm-II cDNA synthesis kit (Promega, Madison, WI), following the manufacturer's instructions. For each reaction the input DNA was 500 ng total RNA. Cytokine and chemokine mRNA expression were examined by probe-based qRT-PCR. The Universal ProbeLibrary Assay Design Center (Roche Diagnostics, Indianapolis, IN) was used to design primers compatible with the Universal ProbeLibrary system, which were subsequently synthesized by Integrated DNA Technologies (Coralville, IA). All probes used were from the Universal ProbeLibrary (Roche Diagnostics, Indianapolis, IN). Primer sequences and probes used in this study are listed in Table 1. A Roche LightCycler 480 Instrument II LC (Roche Diagnostics) was used to quantify all gene expression. Gene-expression levels were calculated using the comparative threshold method and normalized to internal *Gapdh* expression, as determined using the Mouse GAPD Gene Assay (Cat. no. 05046211001; Roche Diagnostics) in multiplex format.

2.7. BALF cytokine measurement

BALF from treatment groups was harvested in 1 ml of PBS containing 2% BSA (Boston BioProducts, Ashland, MA). 50 µl of cell-free BALF was analyzed by cytometric bead array for pro-inflammatory cytokines and chemokines using the Milliplex cytometric bead array kit (Millipore Sigma, Burlington MA Cat # MCYTOMAG-70K-PMX) according to the manufacturer's instructions [38]. The samples were run on a BioRad Bio-Plex 200 system and data was analyzed relative to a 5-parameter logistical standard curve for each corresponding cytokine/chemokine using Bio-Plex Manager 6.1.1.

Table 1
Probe-based qPCR sequences. Sequences for primer pairs used for qPCR mRNA analysis are listed according to gene name. The correspoinding probe used from the Universal Probe Library (Roche) for each primer pair are also listed.

Gene	Primer	Sequence (5′-3′)	Universal Probe Library (Roche) Probe no.
IL4	Sense	CATCGGCATTTTGAACGAG	2
	Anti-sense	CGAGCTCACTCTCTGTGGTG	
IL5	Sense	ACATTGACCGCCAAAAAGAG	97
	Anti-sense	CACCATGGAGCAGCTCAG	
IL9	Sense	GCCTCTGTTTTGCTCTTCAGTT	107
	Anti-sense	GCATTTTGACGGTGGATCAT	
IL10	Sense	CAGAGCCACATGCTCCTAGA	78
	Anti-sense	GTCCAGCTGGTCCTTTGTTT	
IL13	Sense	ACCCAGAGGATATTGCATGG	75
	Anti-sense	TGGGCTACTTCGATTTTGGT	
IL15	Sense	AACAGCTCAGAGAGGTCAGGA	106
	Anti-sense	CCATGAAGAGGCAGTGCTTTT	
IL17a	Sense	TGTGAAGGTCAACCTCAAAGTC	50
	Anti-sense	GAGGGATATCTATCAGGGTCTTCA	
Il33	Sense	CAAAACAAAACTAACAGATTGGTCA	16
	Anti-sense	GACACATTGAGCATCCAAGG	
Eotaxin	Sense	AGAGCTCCACAGCGCTTCT	18
	Anti-sense	GCAGGAAGTTGGGATGGA	
GM-CSF	Sense	GCATGTAGAGGCCATCAAAGA	79
	Anti-sense	CGGGTCTGCACACATGTTA	
MMP-2	Sense	GTGGGACAAGAACCAGATCAC	85
	Anti-sense	GCATCATCCACGGTTTCAG	
MMP-9	Sense	CGACATAGACGGCATCCAG	77
	Anti-sense	CTGTCGGCTGTGGTTCAGT	
MUC5AC	Sense	TCGAGAGGAGCGTTGACAC	22
	Anti-sense	GAGGGTTGCATTGAGGTCAT	
Rantes	Sense	TGCAGAGGACTCTGAGACAGC	110
	Anti-sense	GAGTGGTGTCCGAGCCATA	
TGFβ	Sense	ACGCCAGGAATTGTTGCTAT	56
	Anti-sense	TCAGACATTCGGGAAGCAGT	
GAPD	Sense	AATCTCCACTTTGCCACTGC	GAPDH Assay Probe (Roche)
	Anti-sense	ATGGTGAAGGTCGGTGTGA	

2.8. Statistics

All data were analyzed using GraphPad Prism software (GraphPad Software, La Jolla, CA). See figure legends for specific details.

3. Results

3.1. 5-HT$_{2A}$ receptor activation reduces elevated AHR in chronic OVA allergen challenged BALB/c mice

We first validated that the chronic OVA treatment paradigm resulted in increased enhanced pause (PenH), an index for airway responsiveness, to MeCh using WBP in awake, freely moving mice on Day 147 (48 h post cessation of OVA challenge) (Fig. 2A). We tested the same cohort of mice on Day 152 to assess persistence of hyperresponsiveness (Fig. 2B). The use of PenH to measure bronchoconstriction and pulmonary function is a matter of controversy, largely stemming from numerous conflicting reports regarding the degree of correlation (or lack thereof) of PenH to respiratory resistance [39]. Furthermore, fluctuations in ambient parameters within the plethysmography chamber (i.e. temperature, humidity) can impact airways resistance calculations, thus when utilizing WBP care must be given to minimize environmental variability [40]. Another factor to consider is the type of WBP performed. Whereas data gathered from a sealed chamber plethysmograph (PressureWBP) has been shown to not represent pulmonary resistance, data gathered from a chamber with a

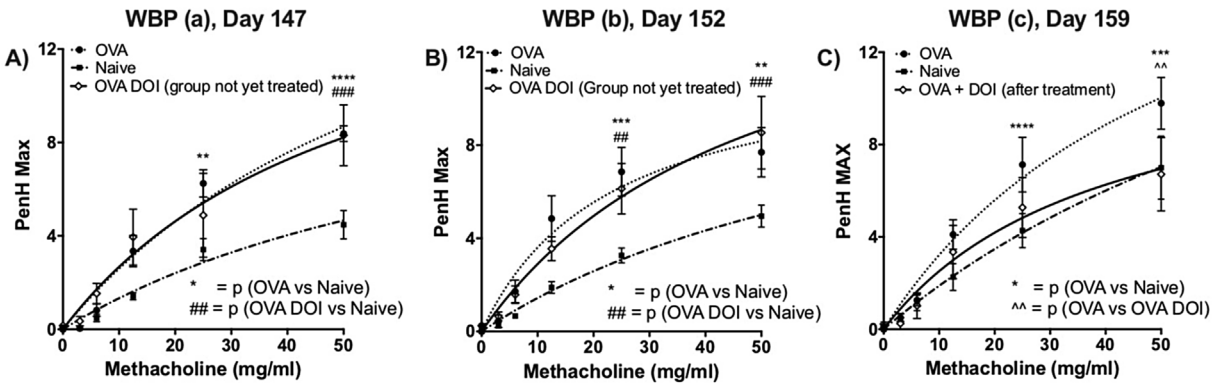

Fig. 2. (*R*)-2,5-Dimethoxy-4-iodoamphetamine [(*R*)-DOI] reduces elevated pulmonary hyperresponsiveness to methacholine. Results are from whole-body plethysmography experiments (WBP) in awake, freely moving mice. (**A.**) Results from WBP experiments 2 days post ovalbumin challenge cessation. (**B.**) Results from WBP prior to ovalbumin challenge (8 days post termination of allergen). (**C**). Naïve mice and those treated nose-only with 1.0 mg/kg (*R*)-DOI for 4 days exhibited significantly reduced airways responsiveness compared to OVA-only treated group. Measurements taken 14 days post cessation of allergen challenge. Data shown is representative of two independent experiments. n = 7–8 animals/treatment group. *p < 0.05 OVA vs Naïve, and ##p < 0.05 OVA DOI vs Naïve (pre-DOI treatment), #p < 0.05 OVA vs OVA + DOI. ^^p < 0.05 OVA vs OVA DOI (post-DOI treatment). Error bars represent ± SE; 2-way ANOVA with Bonferroni post hoc test.

pneumotachograph in its wall (FlowWBP) have been demonstrated to be relevant to pulmonary resistance [41]. Therefore, with proper mathematical analysis and a properly calibrated FlowWBP system (which is the system we utilize), PenH can quantitatively reflect alterations in airway resistance, regardless of any varying ambient parameter [41]. Nevertheless, a certain degree of caution should be observed when considering the use of PenH data from a properly calibrated FlowWBP system as a proxy for direct measurement using forced ventilation to measure pulmonary airways resistance [42].

The observed maximum PenH data from these two time points among groups were not different with respect to PenH response to methacholine and OVA treated animals maintained the same magnitude of change compared to naïve, saline-challenged animals. Starting Day 155 appropriate treatment groups received 1.0 mg/kg inhaled (*R*)-DOI once daily until Day 158. PenH was then measured on Day 159 (14 days post final OVA exposure (Fig. 2C). Mice treated with OVA, and then (*R*)-DOI, displayed reduced PenH responsiveness that was non statistically different from naïve, whereas PenH values in OVA alone-treated animals remained significantly elevated at levels equivalent to the first WBP measurement at 48 h post final OVA exposure. On Day 160 lungs were removed for analysis.

3.2. (R)-DOI reduces mucus overproduction and collagen deposition

Histopathological analysis of lung sections from the different treatment groups revealed that (*R*)-DOI reduced mucus hyperproduction and peribronchial inflammation compared to the OVA alone-treated animals (Figs. 3 and 4). To evaluate collagen deposition and airway fibrosis, adjacent sections were stained with Masson's trichrome. (*R*)-DOI significantly reduced the amount of collagen fibers and smooth muscle layers by 70% (Figs. 5 and 6).

3.3. 5-HT$_{2A}$ receptor activation reverses increased expression of certain proinflammatory markers, and factors involved in collagen deposition

To evaluate the impact of (*R*)-DOI on genes involved in both inflammatory response and collagen deposition [20,22,26,27,33], mRNA from homogenized whole lungs was examined by qRT-PCR. Several genes were identified that were increased in expression by OVA, but whose increases were reduced by (*R*)-DOI: *Il9*, *Il15*, *Gm-csf*, *Muc5ac*, *mmp9*, and *tgf-β* (Fig. 7A–F). Expression of several genes was found to be increased by OVA, but unaffected by (*R*)-DOI: *Il4*, *Il5*, *Rantes*, *Eotaxin* (Fig. 7G–J). Some transcripts were found to be increased by OVA, with levels potentiated by (*R*)-DOI: *Il13*, *Il33* (Fig. 7K–L). Two transcripts were not elevated by the OVA treatment but were elevated subsequent to (*R*)-DOI treatment: *Il10*, *Il17a* (Fig. 7M − N). Two genes tested were unaffected by both OVA and (R)-DOI treatment: *mmp2*, *ifn-γ* (Fig. 7O–P).

To further evaluate the effects of (*R*)-DOI on the cytokine/chemokine profiles of mice chronically challenged with OVA, we performed cytometric bead array analyses on bronchoalveolar lavage fluid (BALF) from naïve, OVA, and OVA + DOI mice. Interestingly, studies by others have demonstrated that repeated OVA exposure can lead to decreases in levels of levels of several acute inflammatory markers [43,44], leading to the hypothesis that it is bronchial smooth muscle remodeling and not inflammation *per-se* that drives airway remodeling and AHR in mouse OVA-induced chronic asthma models. We found significant elevations in the levels of several acute inflammatory markers in the BALF of the OVA treated mice (Fig. 8A–C). (*R*)-DOI treatment produced significant reductions in the levels of GM-CSF and IL-15, but not of RANTES.

4. Discussion

Mice are the most common animal used to model allergic responses in the airways [45], with the BALB/c strain favored because it develops a strong T helper cell 2 (Th2)-biased immunological response following allergen exposure [46]. There are several described acute model paradigms in which sensitized mice are exposed to aerosolized ovalbumin to initiate a pulmonary inflammatory response [47–49]. We previously utilized one of these models when evaluating the effects of 5-HT$_2$ receptor activation to prevent the development of allergic asthma [31]. Unfortunately, acute asthma models in mice have several limitations, including differences in the pattern and distribution of pulmonary inflammation between mice and humans, an absence of *chronic* inflammation, airway structural remodeling, and symptomology that returns to a baseline non-inflamed state within 3–4 days. Because of these limitations, there have been failures to correlate therapeutic efficacy between the acute challenge models (asthma prevention) and clinical trials in humans with already present asthma (rescue of existing symptoms) [46,50].

In order to evaluate the effects of 5-HT$_2$ receptor activation as a therapeutic rather than prophylactic treatment, we sought to develop a model of allergic airways disease in which inflammation and associated symptoms remained present at pathological levels long after the final exposure to allergen. Unfortunately, most asthma-like symptoms in the standard mouse OVA asthma model return to normal/baseline within three-to-four days following the final allergen exposure. Another disadvantage to the standard OVA model is that they do not replicate the

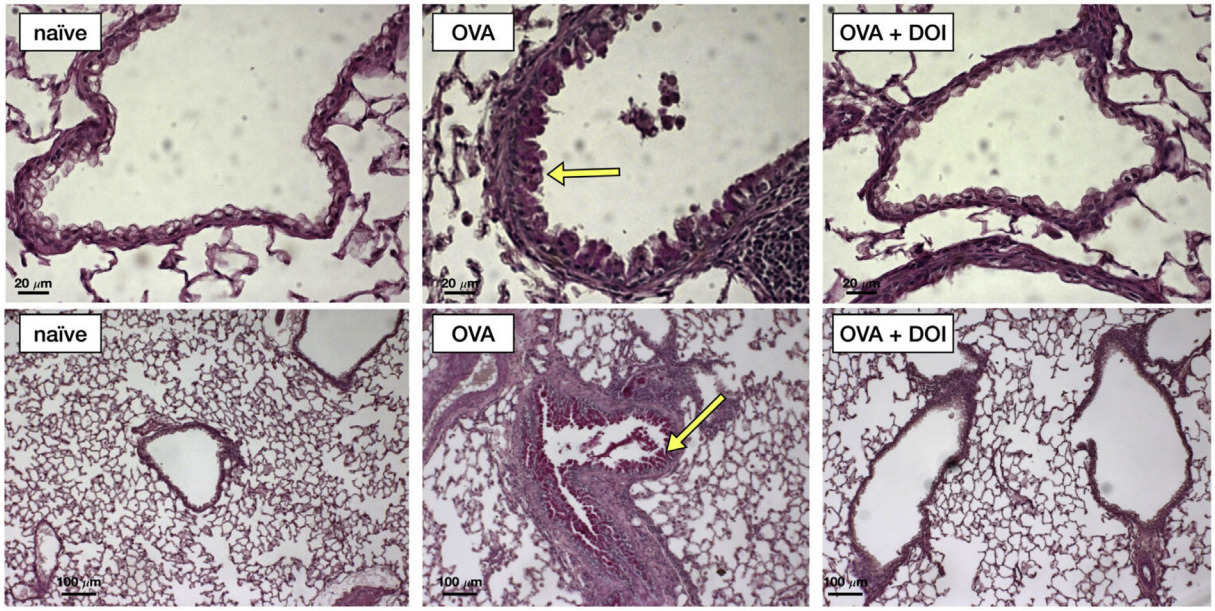

Fig. 3. Chronic OVA-induced peribronchial inflammation and mucus production are reduced following nose-only (*R*)-DOI treatment. Representative sections of airways stained with periodic acid-Schiff (PAS) technique are shown; pink staining denotes mucus (yellow arrows). Naïve saline only treated animals have standard airway morphology with no discernible mucus production. OVA-treated animals have consistently thickened airways with a significant amount of mucus-staining and peribronchial inflammation. Animals treated with 1.0 mg/kg (*R*)-DOI for four consecutive days demonstrate greatly reduced peribronchial inflammation and mucus production. *Upper panels:* 40× objective; *Lower panels*, 10× objective.

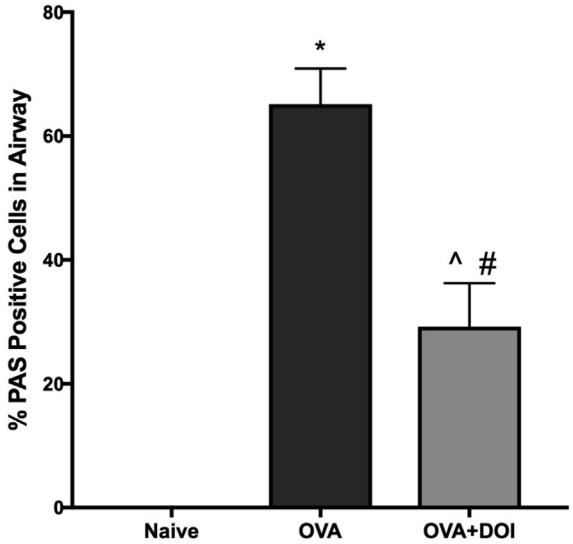

Fig. 4. Inhaled (*R*)-DOI attenuates mucus cell hyperplasia in the airway. The fraction of airways cells producing mucus was quantified following PAS staining. Mucus-producing cells were not observed in naïve animals, whereas OVA-challenge significantly increases number of PAS-positive cells (OVA). Mucus-producing cells are significantly reduced following treatment with 1.0 mg/kg (*R*)-DOI for 4 consecutive days following cessation of allergen challenge (OVA + DOI). P* < 0.0001 vs. Naïve, #P < 0.0001 vs OVA, and P^0.0001 vs Naïve; error bars represent ± SEM; ANOVA with Bonferroni post hoc test. All airways/sections were scored by an unbiased blinded observer for three airways of each animal for three animals each per treatment group (n = 3).

long-term structural changes and airway remodeling that are found in humans with asthma [32]. There are several reports of sub-chronic and chronic allergen exposure models in the literature. However, the symptoms elicited in these models often return to baseline a week or so after the final allergen exposure. Earlier work demonstrated that prolonged exposures to OVA (8 weeks) was able to produce somewhat long-lasting pulmonary inflammation and fibrosis [33] where PenH (a measure of bronchoconstriction) values in response to MeCh remained significantly elevated compared to baseline for up to 2 weeks following the final exposure of OVA, however symptoms began to return to baseline within about one week and were only statically different for up to two weeks. In an attempt to develop a model of prolonged inflammation and AHR at maximal levels without signs of decrease for two weeks and beyond, we added an additional OVA sensitization on Day 17, and extended weekly nasal OVA exposures to 16 weeks. In our protocol, OVA exposures were performed minimally, once weekly, as opposed to three times per week as is used in some sub-chronic paradigms [32,51], in efforts to reduce allergen chronicity [45] because repeated exposure of antigen to airways can result in tolerance to the allergen [52].

In our chronic OVA exposure model, we are able to generate several key symptoms relevant to asthma. These include pulmonary inflammation, mucus overproduction, fibrotic remodeling of airways, and increased PenH responsiveness to methacholine challenge. Importantly, these changes persisted for at least two weeks unabated following the final OVA exposure. At the molecular level, our model demonstrated allergen-induced and persistent increases in expression levels of mRNAs and proteins for several cytokines and chemokines relevant to asthma including GM-CSF, IL5, IL13, Eotaxin, RANTES, and MUC5AC in the lung and BALF. Therefore, our regimen appears to generate an asthma-relevant state in mice that persists for at least several weeks. This persistence and stability of symptomology is important in order to be able to test pharmacotherapies for their ability to rescue and reverse symptoms in a mouse model, something not possible in the traditional acute OVA model or other published chronic models where symptomology is not stable and returns to baseline within two weeks after the final allergen exposure.

Remarkably, a short treatment regimen with the selective 5-HT_2 receptor agonist (*R*)-DOI normalizes pulmonary responsiveness (PenH) to methacholine to control levels. Although we only treated once per day for four days, it may be that fewer treatments are necessary to produce a therapeutic effect. This treatment also resulted in a significant reduction in mucus production, and significantly reduced

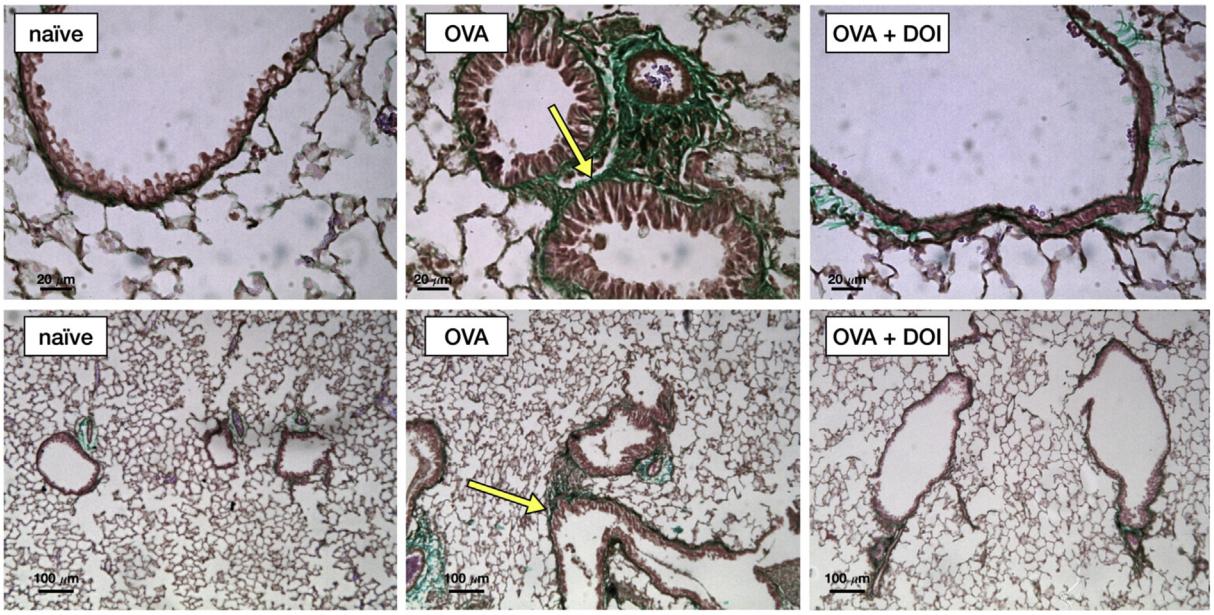

Fig. 5. Collagen deposition around bronchial airways is reduced following 5-HT_{2A} receptor activation. Representative images of Masson's trichrome stained are shown, where green staining around airways represents collagen deposition. Significantly thickened airways with increased collagen staining (yellow arrows) were observed in OVA-treated animals, whereas (*R*)-DOI treated animals exhibited greatly reduced collagen deposits. Saline-treated animals exhibited standard airway morphology. *Upper panels*: 40× objective; *Lower panels:* 10× objective.

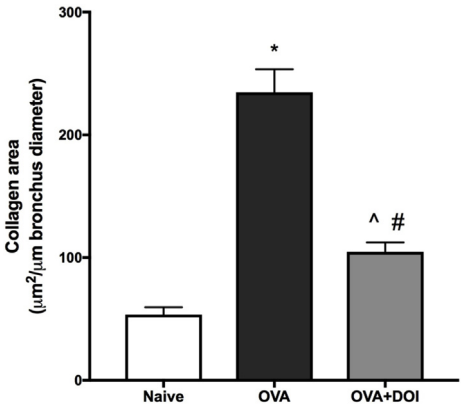

Fig. 6. (*R*)-DOI reduces collagen deposits and peribronchiolar fibrosis in chronic OVA-challenged BALB/c mice. Quantitative analysis of the mean total area of airway fibrosis (μm [2]) per basement membrane length (μm). Fibrotic area around small bronchi was significantly reduced in animals exposed to 1.0 mg/kg (*R*)-DOI for four days (OVA + DOI) compared to OVA-treated animals (OVA). Saline-treated (Naïve) animals exhibited minimal levels of collagen deposition. *P < 0.001 vs Naïve, #P < 0.001 vs OVA, ˆP < 0.05 vs Naïve; error bars represent ± SEM; ANOVA with Bonferroni post hoc test. Morphometric analysis was performed as described in Methods and materials for 10 airways per slide from 6 animals (n = 6) per treatment group.

pulmonary inflammation. The substantial reversal of collagen deposition and fibrosis by (*R*)-DOI suggests that this treatment may also have therapeutic efficacy for other diseases of the lung involving fibrosis like COPD or pulmonary hypertension.

As observed in our previous models of inflammation and asthma, treatment with (*R*)-DOI does not produce a general immunosuppressive effect like steroids. We found that only subsets of key inflammatory markers were reduced. Mechanistically, selective reductions in the expression of GM-CSF, IL-15, and possibly IL-9, may underlie the therapeutic anti-inflammatory effects. These, in combination with reductions in MUC5AC to reduce mucus production [53], and TGF-β and MMP-9 to reduce collagen deposition and fibrosis and increase collagen turnover [54–56], may act together to affect a substantial therapeutic effect to treat pre-existing asthma symptoms and restore the lung to a more normal physiological state. It is important to note these variations occur in message levels of whole-lung homogenate. Further studies are justified not only to evaluate (*R*)-DOI-mediated regulation at the protein level, but also the impact of 5-HT_{2A} activation on individual immune constituent activation, polarization, and recruitment.

Interestingly, reductions in asthma relevant symptoms including inflammation and mucus overproduction in response to treatment with (*R*)-DOI are present despite significant elevations in *Il13* and *Il33* mRNA in the lungs of the OVA + (*R*)-DOI treated mice that are even higher than the OVA alone treated animals. The IL-33/IL-13 axis has been strongly implicated by others in the pathobiology of asthma and other pulmonary diseases. Allergen induced secretion of IL-33 from pulmonary epithelial cells recruits several inflammatory cell types like Th2 cells, eosinophils, and macrophages, that subsequently secrete IL-13, leading to alterations in bronchial smooth muscle contractility, goblet cell metaplasia and mucus overproduction, and airways hyperresponsiveness [57–59]. One explanation for reductions in asthma relevant symptoms in the presence of increased *Il33* and *Il13* gene expression could be that the expression of these genes and their proteins is not always linked to pathological outcomes like goblet cell metaplasia and mucus overproduction. For example, suppression of mitogen activated protein kinase 13 (MAPK13) expression can completely block increases in *Muc5acC* mRNA levels without influencing IL-13 driven processes [60]. 5-HT_{2A} receptor agonists are known to modulate MAPK pathways [61], therefore it is possible that (*R*)-DOI recruits signaling pathways that reduce MAPK activity and subsequent mucin gene expression (e.g. *Muc5ac*), resulting in no goblet cell metaplasia or mucus overproduction without inhibiting broader innate immune processes. Another possibility could be that (R)-DOI is acting directly on 5-HT_2 receptors that target cells for IL-33 (e.g. eosinophils, Th2 cells, macrophages) and having a suppressive effect on their activation and recruitment, despite the presence of IL-33. This second scenario is consistent with our previous studies with an acute OVA model where we observed that (*R*)-DOI prevents recruitment of specific activated Th2 cells and eosinophils to the lung [31]. In accordance with this hypothesis it has been shown that IL-13 and IL-33 form a positive feedback circuit to promote maintenance of a persistent chronic asthma

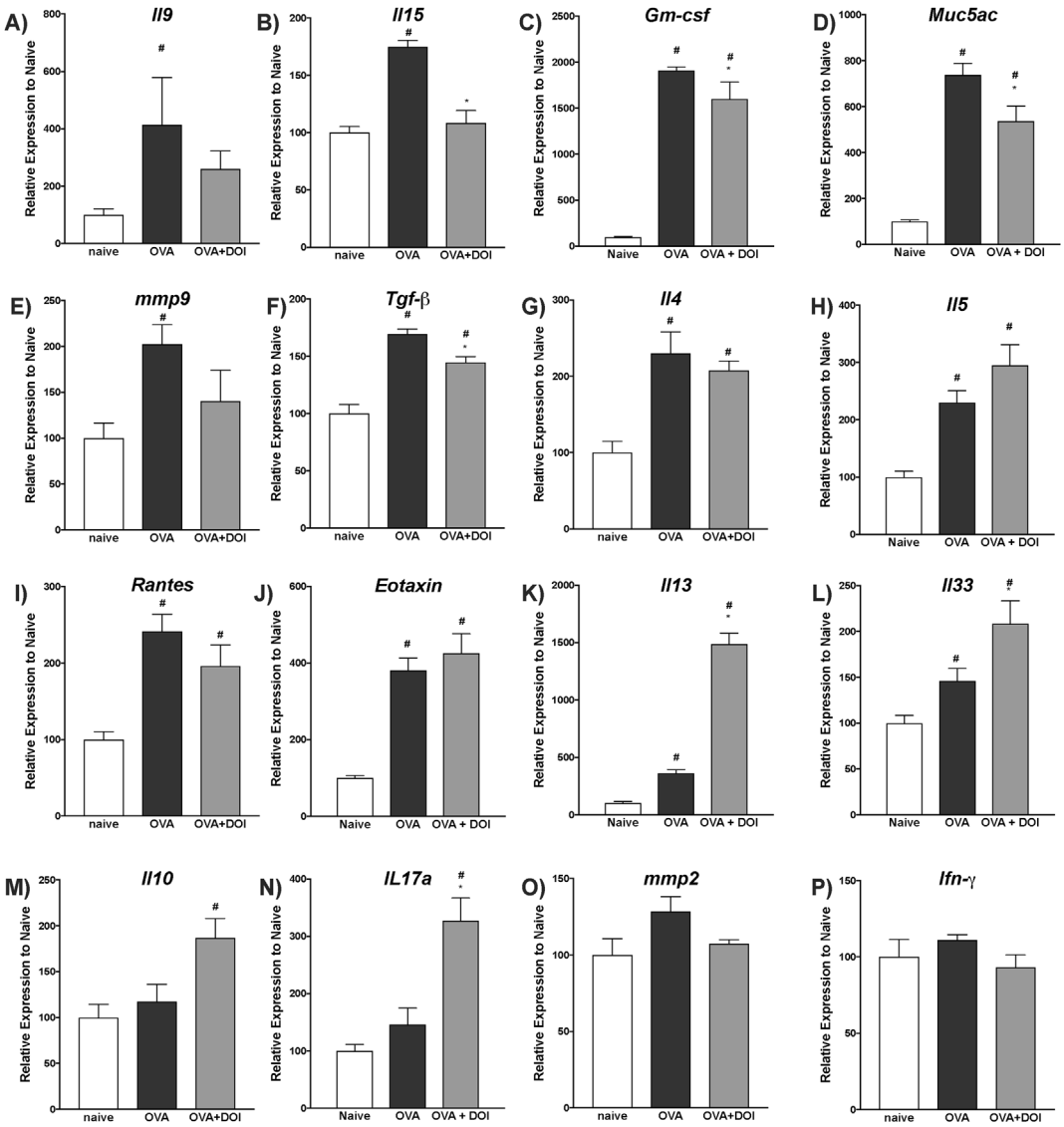

Fig. 7. Inhaled (*R*)-DOI (1.0 mg/kg) induces expression of acute asthmatic factors and reduces expression of collagen deposition factors in the whole lung. Quantitative RT-PCR measurement of mRNA expression levels of numerous inflammatory markers and collagen deposition factors is shown. (*R*)-DOI treatment reduces OVA-induced increase in the mRNA levels of *Il9* (A), *Il15* (B), *Gm-csf* (C), *Muc5ac (D)*, *mmp9* (E), and *TGFβ* (F). Treatment with (R)-DOI has no significant effect on the OVA-induced increases in expression of *Il4* (G), *Il5* (H), *Rantes* (I), and *Eotaxin* (J). (*R*)-DOI treatment increases mRNA expression of *Il13* (K) and *Il33* (L) above levels produced by OVA. OVA does not produce increases in the expression of mRNAs for *Il10* (M), and *Il17a* (N), but their levels are increased by (R)-DOI treatment. Neither OVA nor (*R*)-DOI treatment has any effect on the expression mRNAs for mmp2 (O) or *IFN*γ (P). #P $<$ 0.05 naïve vs. OVA, OVA + DOI; *P $<$ 0.05 OVA vs OVA + DOI; $n = 10$ animals for the naïve group, $n = 5$–6 animals for the OVA group, and $n = 6$–8 animals for the OVA + DOI treatment groups; error bars represent ± SEM; ANOVA with Bonferonni post hoc test.

state [62]. It is possible that the preservation of this axis is a vital component to the success of our chronic model, and its disruption simply does not occur in our limited treatment regimen.

That the majority of inflammatory markers are not reduced by (*R*)-DOI treatment in this chronic model indicates that 5-HT_2 receptor activation primarily leaves the immune system intact and responsive. This is a desirable feature for potential therapeutic strategies based on 5-HT_2 receptor agonism compared to others like steroids and biologics that are known to produce systemic suppression of the immune system and leave the patient vulnerable to opportunistic infections. Further, because the effects of (*R*)-DOI appear to be narrowly directed at blockade of only a subset of inflammatory pathways that together rescue pathology, 5-$HT_{2(A)}$ receptor-based therapeutics for asthma would be steroid sparing, as well as possibly useful for the treatment of severe forms of asthma that are resistant to steroid therapies. As part of this possible mechanism, although 5-$HT_{2(A)}$ receptors are expressed on several types of cells relevant to asthma including bronchial smooth muscle and endothelial cells, macrophages, and eosinophils, there may be differential effects. We previously found that not all types of tissues expressing the target receptor demonstrated an anti-inflammatory response to (*R*)-DOI (Nau et al., 2014). Therefore, the primary site of action may be on smooth muscle and/or endothelial cells to normalize pathology and reverse structural remodeling, whereas effects on other cells like macrophages or neutrophils may be less important and reflected by an observed lack of decreased expression for certain proinflammatory cytokine markers.

In summary, our current work further elucidates the role 5-HT_2 receptors in peripheral tissues. Because expression of the 5-HT_{2A} receptor has been found in airway smooth muscle cells [63], alveolar macrophages [64], activated T-cells [65], and whole lung tissue [30], and our earlier work demonstrated that the anti-inflammatory activity of (*R*)-DOI in other inflammatory models is through functionally

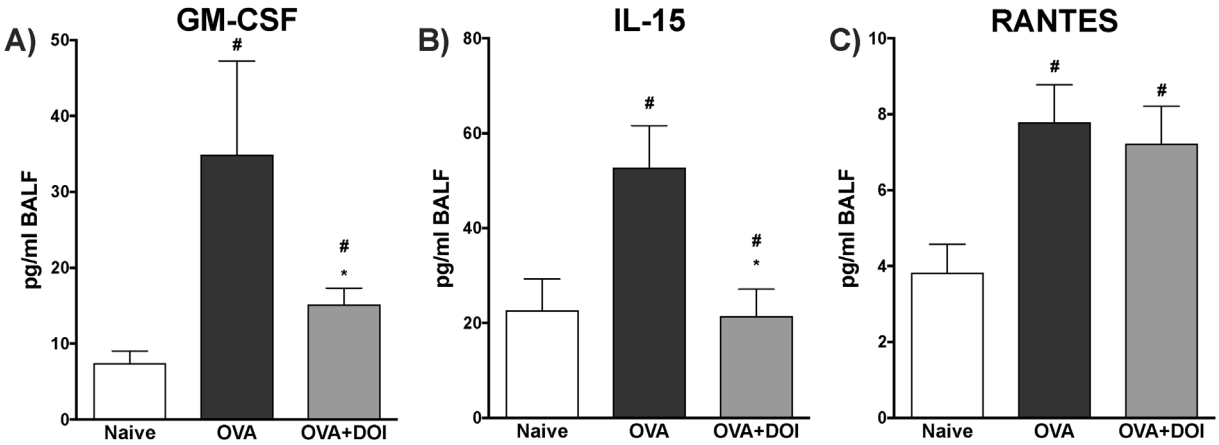

Fig. 8. (*R*)-DOI treatment alters cytokine profile in BALF from chronically-challenged BALB/c mice. BALF was collected from naïve, OVA, and OVA + DOI treated mice and subjected to cytometric bead array analyses. (*R*)-DOI treatment significantly reduced the OVA-induced expression of GM-CSF (A) and IL-15 (B). Levels of RANTES were increased in the OVA treated animals, but (R)-DOI treatment had no effect (C). Cytokine values represent mean values ± SEM for 6–8 animals from the naïve group, 5–7 animals for the OVA group, and 4–6 animals for the OVA + DOI group. Statistical analysis was performed using ANOVA with Bonferonni post hoc test (#P < 0.05 naïve vs. OVA, OVA + DOI; *P < 0.05 OVA vs OVA + DOI).

selective activation of the 5-HT_{2A} receptor [29,30], we hypothesize that the therapeutic effects of (*R*)-DOI in this chronic OVA model are also mediated by 5-HT_{2A} receptor activation. In previous work, we proposed that (*R*)-DOI's site of therapeutic action was directly on pulmonary tissues, in particular activated T-cell populations and innate-immune cells [31], and believe that similar mechanisms are at play here to rescue symptoms of pre-existing asthma. An important feature of our chronic model is the development of fibrosis. We propose that this fibrotic remodeling is reduced by (*R*)-DOI treatment through 5-HT_{2A} receptor mediated suppression of TGF-β and a return to homeostasis of collagen turnover. Together, our data support the further development of 5-$HT_{2(A)}$ receptor activation as a novel therapeutic strategy to treat allergic asthma.

Funding

Support for this work came from an award from the American Asthma Foundation to Charles D. Nichols, the Betsy Gordon Foundation, and a sponsored research contract from Eleusis Benefit Corporation. Stephanie A. Cormier is supported by the National Institutes of Health (NIAID), grant number A1090059.

Author contributions

C.D.N. and S.A.C. - conception and design of research; T.W.F., M.N.S., and C.D.N. -performed experiments; D.B. and T.P.F. - performed cytometric bead analyses. T.W.F. and C.D.N. - analyzed data; T.W.F. and C.D.N. interpreted results of experiments; T.W.F., M.N.S., and C.D.N. - prepared figures; T.W.F. - drafted manuscript; T.W.F., S.A.C. and C.D.N. - edited and revised manuscript; C.D.N. approved final version of manuscript.

Acknowledgements

The authors would like to thank the late Dr. Xiaozhu Huang (Sabre-Sandler Asthma Basic Research Center, San Francisco, CA) for histology sectioning and staining; Dr. F. Nau Jr., for technical advice and assistance; A. Landry, Dr. M. Hibicke, and B. DeVellis for histology analysis and scoring.

Present address of M.N. Sebastian: Vanderbilt University School of Medicine, 2215 Garland Ave., Nashville, TN 37232.

References

[1] D. Hoyer, et al., International union of pharmacology classification of receptors for 5-hydroxytryptamine (serotonin), Pharmacol. Rev. 46 (1994) 157–203.
[2] B.L. Roth, S.A. Berry, W.K. Kroeze, D.L. Willins, K. Kristiansen, Serotonin 5-HT2A receptors: molecular biology and mechanisms of regulation, Crit. Rev. Neurobiol. 12 (1998) 319–338.
[3] N. Santana, A. Bortolozzi, J. Serrats, G. Mengod, F. Artigas, Expression of serotonin1A and serotonin2A receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex, Cerebr. Cortex 14 (2004) 1100–1109, https://doi.org/10.1093/cercor/bhh070 (New York, N.Y. 1991).
[4] D.E. Nichols, M.W. Johnson, C.D. Nichols, Psychedelics as medicines: an emerging new paradigm, Clin. Pharmacol. Ther. 101 (2017) 209–219, https://doi.org/10.1002/cpt.557.
[5] J.C. Beique, M. Imad, L. Mladenovic, J.A. Gingrich, R. Andrade, Mechanism of the 5-hydroxytryptamine 2A receptor-mediated facilitation of synaptic activity in prefrontal cortex, Proc. Natl. Acad. Sci. U. S. A 104 (2007) 9870–9875, https://doi.org/10.1073/pnas.0700436104.
[6] D.A. Martin, C.D. Nichols, Psychedelics recruit multiple cellular types and produce complex transcriptional responses within the brain, EBioMedicine 11 (2016) 262–277, https://doi.org/10.1016/j.ebiom.2016.08.049.
[7] G. Zhang, R.W. Stackman Jr., The role of serotonin 5-HT2A receptors in memory and cognition, Front. Pharmacol. 6 (2015) 225, https://doi.org/10.3389/fphar.2015.00225.
[8] J. Gonzalez-Maeso, et al., Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior, Neuron 53 (2007) 439–452, https://doi.org/10.1016/j.neuron.2007.01.008.
[9] D.E. Nichols, Psychedelics. *Pharmacological reviews* 68 (2016) 264–355, https://doi.org/10.1124/pr.115.011478.
[10] C.D. Nichols, Serotonin 5-HT(2A) receptor function as a contributing factor to both neuropsychiatric and cardiovascular diseases, Cardiovascular psychiatry and neurology (2009) 475108, https://doi.org/10.1155/2009/475108 2009.
[11] T. Machida, K. Iizuka, M. Hirafuji, 5-hydroxytryptamine and its receptors in systemic vascular walls, Biol. Pharm. Bull. 36 (2013) 1416–1419.
[12] G. Cerulo, et al., Serotonin activates cell survival and apoptotic death responses in cultured epithelial thyroid cells, Biochimie 105 (2014) 211–215, https://doi.org/10.1016/j.biochi.2014.06.020.
[13] M. Gooz, P. Gooz, L.M. Luttrell, J.R. Raymond, 5-HT2A receptor induces ERK phosphorylation and proliferation through ADAM-17 tumor necrosis factor-alpha-converting enzyme (TACE) activation and heparin-bound epidermal growth factor-like growth factor (HB-EGF) shedding in mesangial cells, J. Biol. Chem. 281 (2006) 21004–21012, https://doi.org/10.1074/jbc.M512096200.
[14] T.C. Pellegrino, B.M. Bayer, Role of central 5-HT(2) receptors in fluoxetine-induced decreases in T lymphocyte activity, Brain Behav. Immun. 16 (2002) 87–103, https://doi.org/10.1006/brbi.2001.0625.
[15] D.J. Welsh, M. Harnett, M. MacLean, A.J. Peacock, Proliferation and signaling in fibroblasts: role of 5-hydroxytryptamine2A receptor and transporter, Am. J. Respir. Crit. Care Med. 170 (2004) 252–259, https://doi.org/10.1164/rccm.200302-264OC.
[16] R.G. Ruddell, et al., A role for serotonin (5-HT) in hepatic stellate cell function and liver fibrosis, Am. J. Pathol. 169 (2006) 861–876, https://doi.org/10.2353/ajpath.2006.050767.
[17] J. Stefulj, B. Jernej, L. Cicin-Sain, I. Rinner, K. Schauenstein, mRNA expression of serotonin receptors in cells of the immune tissues of the rat, Brain Behav. Immun. 14 (2000) 219–224, https://doi.org/10.1006/brbi.1999.0579.
[18] B.N. Kang, et al., Regulation of serotonin-induced trafficking and migration of

eosinophils, PLoS One 8 (2013) e54840, , https://doi.org/10.1371/journal.pone.0054840.
[19] P. Segura, et al., Role of 5-HT2A, 5-HT4 and 5-HT7 receptors in the antigen-induced airway hyperresponsiveness in Guinea-pigs, Clin. Exp. Allergy : J. British. Soc. Allergy. Clin. Immunol. 40 (2010) 327–338, https://doi.org/10.1111/j.1365-2222.2009.03412.x.
[20] W.W. Busse, R.F. Lemanske Jr., Asthma. *The New England journal of medicine* 344 (2001) 350–362, https://doi.org/10.1056/nejm200102013440507.
[21] S.R. Naik, S.M. Wala, Inflammation, allergy and asthma, complex immune origin diseases: mechanisms and therapeutic agents, Recent Pat. Inflamm. Allergy Drug Discov. 7 (2013) 62–95.
[22] Q. Hamid, M. Tulic, Immunobiology of asthma, Annu. Rev. Physiol. 71 (2009) 489–507, https://doi.org/10.1146/annurev.physiol.010908.163200.
[23] A. Jatakanon, et al., Neutrophilic inflammation in severe persistent asthma, Am. J. Respir. Crit. Care Med. 160 (1999) 1532–1539, https://doi.org/10.1164/ajrccm.160.5.9806170.
[24] N.G. Carroll, S. Mutavdzic, A.L. James, Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma, Thorax 57 (2002) 677–682.
[25] C.M. Lloyd, E.M. Hessel, Functions of T cells in asthma: more than just T(H)2 cells, Nat. Rev. Immunol. 10 (2010) 838–848, https://doi.org/10.1038/nri2870.
[26] P.J. Barnes, Pathophysiology of allergic inflammation, Immunol. Rev. 242 (2011) 31–50, https://doi.org/10.1111/j.1600-065X.2011.01020.x.
[27] J. Deckers, F. Branco Madeira, H. Hammad, Innate immune cells in asthma, Trends Immunol. 34 (2013) 540–547, https://doi.org/10.1016/j.it.2013.08.004.
[28] T.W. Flanagan, C.D. Nichols, Psychedelics as anti-inflammatory agents, Int. Rev. Psychiatry (2018) 1–13, https://doi.org/10.1080/09540261.2018.1481827.
[29] B. Yu, et al., Serotonin 5-hydroxytryptamine(2A) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency, J. Pharmacol. Exp. Ther. 327 (2008) 316–323, https://doi.org/10.1124/jpet.108.143461.
[30] F. Nau Jr., B. Yu, D. Martin, C.D. Nichols, Serotonin 5-HT2A receptor activation blocks TNF-alpha mediated inflammation in vivo, PLoS One 8 (2013) e75426, , https://doi.org/10.1371/journal.pone.0075426.
[31] F. Nau Jr.et al., Serotonin 5-HT(2) receptor activation prevents allergic asthma in a mouse model, Am. J. Physiol. Lung Cell Mol. Physiol. 308 (2015) L191–L198, https://doi.org/10.1152/ajplung.00138.2013.
[32] N.R. Locke, S.G. Royce, J.S. Wainewright, C.S. Samuel, M.L. Tang, Comparison of airway remodeling in acute, subacute, and chronic models of allergic airways disease, American journal of respiratory cell and molecular biology 36 (2007) 625–632, https://doi.org/10.1165/rcmb.2006-0083OC.
[33] C. Koerner-Rettberg, S. Doths, A. Stroet, J. Schwarze, Reduced lung function in a chronic asthma model is associated with prolonged inflammation, but independent of peribronchial fibrosis, PLoS One 3 (2008) e1575, https://doi.org/10.1371/journal.pone.0001575.
[34] E. Hamelmann, et al., Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography, Am. J. Respir. Crit. Care Med. 156 (1997) 766–775, https://doi.org/10.1164/ajrccm.156.3.9606031.
[35] D. You, et al., Inchoate CD8+ T cell responses in neonatal mice permit influenza-induced persistent pulmonary dysfunction, J. Immunol. 181 (2008) 3486–3494 (Baltimore, Md. : 1950).
[36] J.D. Puerta-Arias, P.A. Pino-Tamayo, J.C. Arango, A. Gonzalez, Depletion of neutrophils promotes the resolution of pulmonary inflammation and fibrosis in mice infected with paracoccidioides brasiliensis, PLoS One 11 (2016) e0163985, , https://doi.org/10.1371/journal.pone.0163985.
[37] C.L. Stumm, et al., Lung remodeling in a mouse model of asthma involves a balance between TGF-beta1 and BMP-7, PLoS One 9 (2014) e95959, , https://doi.org/10.1371/journal.pone.0095959.
[38] L.R. Buckner, M.E. Lewis, S.J. Greene, T.P. Foster, A.J. Quayle, Chlamydia trachomatis infection results in a modest pro-inflammatory cytokine response and a decrease in T cell chemokine secretion in human polarized endocervical epithelial cells, Cytokine 63 (2013) 151–165, https://doi.org/10.1016/j.cyto.2013.04.022.
[39] J. Bates, et al., The use and misuse of Penh in animal models of lung disease, American journal of respiratory cell and molecular biology 31 (2004) 373–374.
[40] G. Enhorning, S. van Schaik, C. Lundgren, I. Vargas, Whole-body plethysmography, does it measure tidal volume of small animals? Can. J. Physiol. Pharmacol. 76 (1998) 945–951.
[41] M. Lomask, Further exploration of the Penh parameter, Exp. Toxicol. Pathol. : official journal of the Gesellschaft fur Toxikologische Pathologie 57 (Suppl 2) (2006) 13–20, https://doi.org/10.1016/j.etp.2006.02.014.
[42] K.A. Verheijden, P.A. Henricks, F.A. Redegeld, J. Garssen, G. Folkerts, Measurement of airway function using invasive and non-invasive methods in mild and severe models for allergic airway inflammation in mice, Front. Pharmacol. 5 (2014) 190, https://doi.org/10.3389/fphar.2014.00190.
[43] J.R. Johnson, et al., Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling, Am. J. Respir. Crit. Care Med. 169 (2004) 378–385, https://doi.org/10.1164/rccm.200308-1094OC.
[44] C. Doras, et al., Lung responses in murine models of experimental asthma: value of house dust mite over ovalbumin sensitization, Respir. Physiol. Neurobiol. 247 (2018) 43–51, https://doi.org/10.1016/j.resp.2017.09.001.
[45] G.R. Zosky, P.D. Sly, Animal models of asthma, Clin. Exp. Allergy : J. British. Soc. Allergy. Clin. Immunol. 37 (2007) 973–988, https://doi.org/10.1111/j.1365-2222.2007.02740.x.
[46] A.T. Nials, S. Uddin, Mouse models of allergic asthma: acute and chronic allergen challenge, Disease models & mechanisms 1 (2008) 213–220, https://doi.org/10.1242/dmm.000323.
[47] G. Grunig, et al., Requirement for IL-13 independently of IL-4 in experimental asthma, Science (New York, N.Y.) 282 (1998) 2261–2263.
[48] T. Morokata, J. Ishikawa, T. Yamada, Antigen dose defines T helper 1 and T helper 2 responses in the lungs of C57BL/6 and BALB/c mice independently of splenic responses, Immunol. Lett. 72 (2000) 119–126.
[49] R.K. Kumar, C. Herbert, P.S. Foster, The "classical" ovalbumin challenge model of asthma in mice, Curr. Drug Targets 9 (2008) 485–494.
[50] M.V. Aun, R. Bonamichi-Santos, F.M. Arantes-Costa, J. Kalil, P. Giavina-Bianchi, Animal models of asthma: utility and limitations, J. Asthma Allergy 10 (2017) 293–301, https://doi.org/10.2147/jaa.s121092.
[51] S. Fernandez-Rodriguez, W.R. Ford, K.J. Broadley, E.J. Kidd, Establishing the phenotype in novel acute and chronic murine models of allergic asthma, Int. Immunopharmacol. 8 (2008) 756–763, https://doi.org/10.1016/j.intimp.2008.01.025.
[52] M. Ostroukhova, et al., Tolerance induced by inhaled antigen involves CD4(+) T cells expressing membrane-bound TGF-beta and FOXP3, The Journal of clinical investigation 114 (2004) 28–38, https://doi.org/10.1172/jci20509.
[53] C.M. Evans, et al., The polymeric mucin Muc5ac is required for allergic airway hyperreactivity, Nat. Commun. 6 (2015) 6281, https://doi.org/10.1038/ncomms7281.
[54] Y. Ma, et al., Immunization against TGF-beta1 reduces collagen deposition but increases sustained inflammation in a murine asthma model, Hum. Vaccines Immunother. 12 (2016) 1876–1885, https://doi.org/10.1080/21645515.2016.1145849.
[55] M. Hoshino, M. Takahashi, Y. Takai, J. Sim, Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma, J. Allergy Clin. Immunol. 104 (1999) 356–363.
[56] T. Kobayashi, et al., Matrix metalloproteinase-9 activates TGF-beta and stimulates fibroblast contraction of collagen gels, Am. J. Physiol. Lung Cell Mol. Physiol. 306 (2014) L1006–L1015, https://doi.org/10.1152/ajplung.00015.2014.
[57] D.E. Byers, et al., Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease, The Journal of clinical investigation 123 (2013) 3967–3982, https://doi.org/10.1172/jci65570.
[58] C. Donovan, J.E. Bourke, R. Vlahos, Targeting the IL-33/IL-13 Axis for respiratory viral infections, Trends Pharmacol. Sci. 37 (2016) 252–261, https://doi.org/10.1016/j.tips.2016.01.004.
[59] H. Makrinioti, M. Toussaint, D.J. Jackson, R.P. Walton, S.L. Johnston, Role of interleukin 33 in respiratory allergy and asthma, The Lancet. Respiratory medicine 2 (2014) 226–237, https://doi.org/10.1016/s2213-2600(13)70261-3.
[60] Y.G. Alevy, et al., IL-13-induced airway mucus production is attenuated by MAPK13 inhibition, The Journal of clinical investigation 122 (2012) 4555–4568, https://doi.org/10.1172/jci64896.
[61] A. Szabo, A. Kovacs, E. Frecska, E. Rajnavolgyi, Psychedelic N,N-dimethyltryptamine and 5-methoxy-N,N-dimethyltryptamine modulate innate and adaptive inflammatory responses through the sigma-1 receptor of human monocyte-derived dendritic cells, PLoS One 9 (2014) e106533, , https://doi.org/10.1371/journal.pone.0106533.
[62] C.A. Christianson, et al., Persistence of asthma requires multiple feedback circuits involving type 2 innate lymphoid cells and IL-33, J. Allergy Clin. Immunol. 136 (2015) 59–68, https://doi.org/10.1016/j.jaci.2014.11.037 e14.
[63] Y. Bai, M. Zhang, M.J. Sanderson, Contractility and Ca2+ signaling of smooth muscle cells in different generations of mouse airways, American journal of respiratory cell and molecular biology 36 (2007) 122–130, https://doi.org/10.1165/rcmb.2006-0036OC.
[64] Z. Mikulski, et al., Serotonin activates murine alveolar macrophages through 5-HT2C receptors, Am. J. Physiol. Lung Cell Mol. Physiol. 299 (2010) L272–L280, https://doi.org/10.1152/ajplung.00032.2010.
[65] M. Leon-Ponte, G.P. Ahern, P.J. O'Connell, Serotonin provides an accessory signal to enhance T-cell activation by signaling through the 5-HT7 receptor, Blood 109 (2007) 3139–3146, https://doi.org/10.1182/blood-2006-10-052787.