Effectiveness of Brilaroxazine on Functional and Underlying Pathological Inflammation and Fibrosis Parameters in a Bleomycin-induced Idiopathic Pulmonary Fibrosis in Male Sprague Dawley Rats

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https://bit.ly/ATSIPF

Abstract # 15059 | Poster # P169

ATS International Conference in San Diego, May 16-22, 2024

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and debilitating lung disease with persistent alveolar inflammation and fibrosis.[1] Its epidemiology involves a global prevalence of ~3 million, a median survival time of two to five years, and 50,000 deaths annually in the U.S. This condition's pathology involves increased pulmonary serotonin (5-HT) 2A/2B/7 receptor expression.[2]

Serotonin (5-hydroxytryptamine; 5-HT) plays a significant role in IPF's pathophysiology. Systemically, this neuroendocrine hormone exerts both a vasoactive effect on pulmonary arteries and stimulation of lung myofibroblast actions.[3,4] Pulmonary 5-HT mediates its effects through 5-$HT_{2A/2B/7}$ receptors.[5-7] Rodent studies of bleomycin-induced pulmonary fibrosis indicate that modulation of these receptors, as seen with terguride (a 5-$HT_{2A/2B}$ antagonist), SB-215505 (5-HT_{2B} receptor antagonist), and SB-269970 (a 5-HT_7 receptor antagonist).[8-10] Pharmacology work with SB-269970 indicates that this target may be integral to inflammation and fibrosis.[10-12]

Brilaroxazine (RP5063) is a novel multimodal serotonin (5-HT) and dopamine (D) receptor modulator that mitigates multiple inflammatory cytokines.[13-21] It specifically functions as a $D_{2/3/4}$ and 5-$HT_{1A/2A}$ partial agonist and a 5-$HT_{2B/7}$ antagonist with binding affinity for 5-HT_{2B} > D_2.[13-16] Brilaroxazine is a potent inhibitor with functional antagonist activity for 5-HT_{2B} and 5-HT_7 receptors (binding affinities [Ki]) 0.19nM and 2.7nM, respectively.[13,18,19] This agent brings an established efficacy, pharmacokinetic, and safety profile in Phase 1-3 clinical studies.[13-15, 22]

Brilaroxazine's effects on vascular fibrosis (5-HT_{2B} receptor), proliferation (5-$HT_{2A/2B}$ receptor), relaxation (5-HT_{2A} receptor), and inflammation (5-HT_7 receptor) led to interest in this agent as a treatment option for IPF.[18,19,23] Recent work utilizing a bleomycin-induced (BLM) model validated brilaroxazine's impact on survival, functional, histologic, and pathophysiological parameters (including inflammation and extracellular deposition).[24,25] Such findings suggest that its effects on multiple 5-HT targets account for these findings and have prompted a follow-up study to validate its prior IPF efficacy results and clarify its pharmacologic actions in mediating disease pathology.[24,25]

OBJECTIVE

This study aimed to evaluate the efficacy of brilaroxazine, SB-269970, and nintedanib in bleomycin-induced IPF in male Sprague Dawley rats. Specific intents included the validation of 1) brilaroxazine's previous efficacy data in the bleomycin-induced IPF model, 2) involvement of 5-$HT_{2B/7}$ in IPF's pathobiology by comparing 5-HT_7 antagonist and nintedanib, a tyrosine kinase inhibitor, and 3) IPF efficacy by comparing the positive control nintedanib, an approved treatment for this condition

METHODS

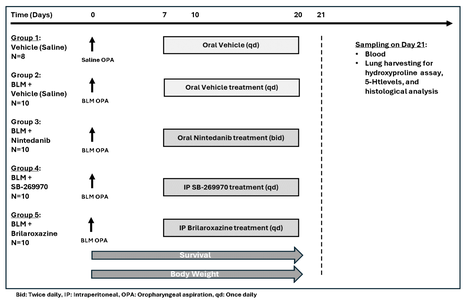
Regulatory

Animal studies occurred at an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) facility and in compliance with Institutional Animal Care and Use Committees (IACUC) protocols.

Procedures

The study followed guidance for bleomycin (BLM)-induction of IPF in SD rats.[10,24-26] The investigation involved 48 adult male Sprague Dawley (SD) rats (Charles River, Raleigh, NC), randomized to one of five groups: 1) Vehicle (Saline) 10mL/kg, *p.o., q.d.* (n=8); 2) BLM + Vehicle (Saline) 10mL/kg, *p.o., q.d.* (n=10); 3) BLM + Nintedanib 60 mg/kg, 10mL/kg, *p.o., b.i.d.* (n=10); 4) BLM + SB-269970 1 mg/kg, 10mL/kg, *i.p., q.d.* (n=10); and 5) BLM + Brilaroxazine: Group 5, 15 mg/kg, 10mL/kg, *i.p., b.i.d.*

Figure 1 Study Schema



Bid: Twice daily, IP: Intraperitoneal, OPA: Oropharyngeal aspiration, qd: Once daily

Assessments and Analysis

The primary outcomes were survival and weight. Blood collection occurred under anesthesia on Day 21. Subsequently, harvesting, weighing, and preparation of the whole lung (and sections) followed animal euthanization via CO_2 fixation.

Lung sample evaluation included hydroxyproline and 5-HT levels, as well as histological analysis.

- Hydroxyproline levels involved color change measurement in spectrophotometry at 55 nm of oxidized samples with Chloramine-T and reacting with Ehrlich's reagent.
- Histology involved hematoxylin and eosin [H&E] and Masson's trichrome staining. Fibrosis grading utilized Ashcroft fibrosis scoring. Leica Analysis software (Leica Microsystems; Deerfield, IL) calculated collagen proportionate areas (%CPA) with Masson's trichrome staining.
- Immunohistochemical (IHC) analysis of prepared left lung sections focused on COL1a1 (collagen protein) and TGF β-1 (growth factor) using primary antibodies and a ready-to-use IHC kit.

Data descriptions were mean ± SEM. Statistical evaluations were made, including comparisons Vs. the non-induced vehicle (for the non-BLM-induced group) and the BLM-induced vehicle (for treatments). Statistical comparisons involved ANOVA (then Dunnett's multiple comparisons post hoc test for BLM). A p-value of <0.05 was significant.

DISCUSSION

This study is the second preclinical evaluation of brilaroxazine's effects on IPF. It validates prior work with this agent using the BLM model.[24,25] Its design and execution were consistent with standard models.[10,24-27]

Treatment stemmed this pathological process. The nintedanib, SB-269970, and brilaroxazine groups (3-5) exhibited a considerable reduction in both fibrosis score and collagen accumulation within the lungs and a noteworthy decrease in the expression of COL1a1 and TGF β-1, in comparison to the BLM + veh group (2).

This study demonstrates that brilaroxazine impacts survival, body weight, lung weight, and underlying fibrosis pathology. It shows a similar or better effect seen with the positive controls, nintedanib and SB-269970. Nintedanib (Ofev), an inhibitor of tyrosine kinases, is an FDA-approved treatment for IPF. SB-269970, a research compound, is a potent 5-HT_7 receptor antagonist. The similarity of effects with SB-269970 reflects the influence of both agents on the 5-HT_7 receptor in mediating the inflammatory and fibrotic pathological process.[8-12,24,25]

This finding reinforces and validates prior findings with brilaroxazine in mortality and functionality, plus inflammation and fibrosis.[24,25] The prior brilaroxazine study showed positive effects on survival, body weight, lung edema, fibrogenic cytokine production, hydroxyproline content, respiratory resistance, and cardiopulmonary capacity, with the initial treatment at Day 1 a little more effective than intervention at Day 10.

Multiple effects via 5-$HT_{2B/7}$ may account for these effects with brilaroxazine.[28-30] Other 5-HT_{2B} receptor antagonists have demonstrated such actions in IPF.[29,30] The decrease in BLM-induced up-regulated hydroxyproline content supports brilaroxazine's antifibrotic role in this current and the prior study.[24,25] Also, this agent attenuates multiple cytokines (IL-6, INF-γ [IP10], MIP1, MCP1, RANTES) in its prior IPF study and other works in pulmonary arterial hypertension and psoriasis, supporting its anti-inflammatory action. [18,19, 23-25, 31] The 5-HT signaling pathology leads to broncho- and pulmonary arterial constriction, myofibroblast, and smooth muscle cell hypertrophic and hyperplastic alterations.[28-30,32] These effects involve myofibroblast inflammation, proliferation, fibrogenesis involving extracellular matrix deposition, and pulmonary vascular endothelial and smooth muscle cell effects.[18,19,28-30,32]

CONCLUSION

Brilaroxazine intervention attenuated the effects of BLM induction on multiple parameters, notably body weight, lung weight, and hydroxyproline level. It exhibited a considerable attenuation in both fibrosis score, collagen accumulation, and COL1a1 and TGF β-1 expression due to BLM induction. Brilaroxazine's effects appeared similar (and in some cases better than) the positive controls, SB-269970 and nintedanib. Its interactions with multiple 5-HT targets, particularly 5-$HT_{2B/7}$ receptors, within the pulmonary tissue, provide an underlying basis for this agent's effectiveness. Brilaroxazine, via its actions on fibrosis and inflammation involving the pulmonary vasculature and myofibroblasts via 5-$HT_{2B/7}$, significantly improved key endpoints and pathological biomarkers in this BLM-induced IPF model in SD rats. The findings from this work validate prior investigation with brilaroxazine in an IPF rat model and involvement of 5-$HT_{2B/7}$ receptors in the pathobiology of IPF.

ACKNOWLEDGEMENTS & DISCLOSURES

Disclosures:

Laxminarayan Bhat, Ph.D. and Seema R Bhat, M.Sc. are employees of Reviva Pharmaceuticals, Inc.

Acknowledgements:

John M. York, PharmD, MBA, of Akita Biomedical, Inc. provided editorial support, and Reviva Pharmaceuticas, Inc. funded this effort.

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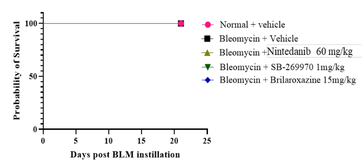
RESULTS

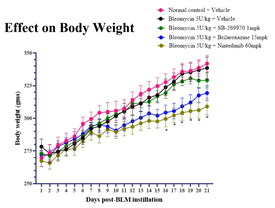
Figure 2

Figure 2. Survival (A) and Body Weight (B) through Treatment, and Absolute Lung (C) Weight on Day 21 in the IPF Rat Model

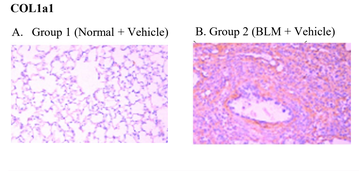
2A Treatment Effect on Survival



2B Effect on Body Weight



2C Treatment Effect on Lung Weight

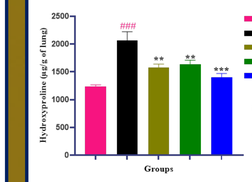


*###p <0.001 Vs Normal control, *p<0.05, ***p<0.001 Vs BLM + Vehicle.*

Figure 2A reflects the survival out to Day 20 as 100% in all groups. Figure 2B shows lower body weight gains in the BLM-induction and treatment groups (3-5) Vs. both vehicle control groups (1 and 2). All treatments were significant vs. the normal control (p<0.001), and only BLM + nintedanib (Group 3) was significant (p<0.05) vs. the BLM + vehicle (Group 2). Figure 2C reflects a significant lung weight increase on Day 21 for the BLM + vehicle (Group 2) vs. the normal control (Group 1) (p<0.001). vs. BLM + vehicle, all treatment groups (2-5) limited this increase significantly, with BLM + nintedanib (Group 3) and BLM + brilaroxazine (Group 5) (p<0.001) and BLM + SB-269970 (p<0.05)

Figure 3

Figure 3. Lung Hydroxyproline Levels at Day 21 in the IPF Rat Model

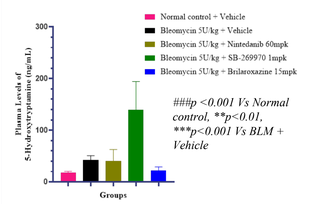


*###p <0.001 Vs Normal control, **p<0.01, ***p<0.001 Vs BLM + Vehicle.*

Figure 3 shows significant hydroxyproline levels increase for the BLM + vehicle (Group 2) vs. the normal control (Group 1) (p<0.001). Vs. BLM + vehicle, all treatment groups (2-5) limited this increase significantly, with BLM + nintedanib (Group 3) and BLM + SB-269970 (Group 4) (p<0.01), and BLM + brilaroxazine (Group 5) (p<0.001)

Figure 4

Figure 4. 5-HT Levels at Day 21 in the IPF Rat Model



*###p <0.001 Vs Normal control, **p<0.01, ***p<0.001 Vs BLM + Vehicle*

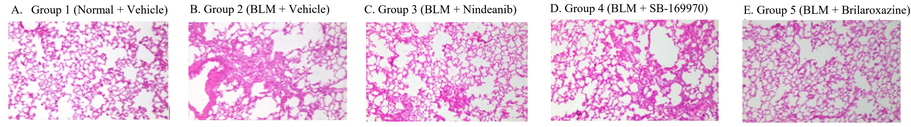
All induced groups had higher 5-HT plasma levels vs. the normal vehicle (N.S.). BLM induction produced a two-fold increase in 5-HT levels vs. normal vehicles (N.S.). Compared to BLM + vehicle, treatments are not significant, though the SB-269970 displays much higher (due to four exceedingly high samples) and brilaroxazine lower levels (by half) in magnitude.

Figure 5

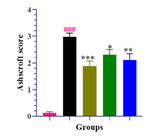
Figure 5. Histological Changes Displayed by H&E Staining (A-E) of Lung Tissue and Ashcroft Fibrosis Score (F), and Masson's Trichrome Staining (G-K) and % Collagen Proportionate Area (L) in the IPF Rat Model on Day 21.

H&E Staining and Ashcroft Fibrosis Score

A. Group 1 (Normal + Vehicle) B. Group 2 (BLM + Vehicle) C. Group 3 (BLM + Nindeanib) D. Group 4 (BLM + SB-169970) E. Group 5 (BLM + Brilaroxazine)



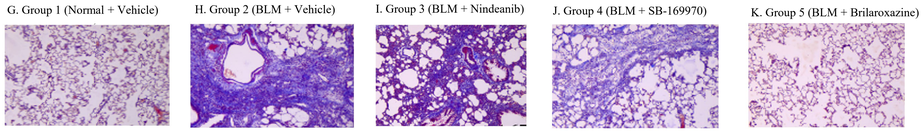
F. Ashcroft Fibrosis Scores



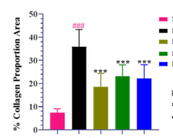
###p <0.001 Vs. Normal control. *p<0.05, **p<0.01, ***p<0.001, Vs BLM + Vehicle

Masson's Trichrome Staining and % Collagen Proportionate Area

G. Group 1 (Normal + Vehicle) H. Group 2 (BLM + Vehicle) I. Group 3 (BLM + Nindeanib) J. Group 4 (BLM + SB-169970) K. Group 5 (BLM + Brilaroxazine)



L. % Collagen Proportionate Area



###p <0.001 Vs. Normal control. *p<0.05, **p<0.01, ***p<0.001, Vs BLM + Vehicle

Concerning lung fibrosis due to BLM-induction and mitigation by the three treatment options (Groups 3-5), H&E staining (Figure 5A-E) and Masson's Trichrome staining (Figures 5G-K) display notable pathologic development. The Ashcroft score (Figure 5F) reflects a significant increase in the BLM + Vehicle group Vs. the normal control (p<0.001). Treatments show significant attenuation of this fibrotic development, with BLM + Nitedanib (p<0.001), BLM + brilaroxazine (p<0.01), and BLM + SB-26970 (p<0.05). The % collagen proption area analysis reflects a significant (p<0.001) increase in fibrosis in lung tissue. All treatments display a significant attenuation of fibrosis (p<0.001).

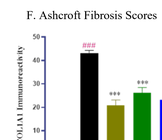
Figure 6

Figure 6. Histological Changes Displayed by IHC Staining and Immunoreactivity Expression of Lung Tissue Specific for COL1a1 (A-F) and TGF β-1 (G-L) in the IPF Rat Model on Day 21.

COL1a1

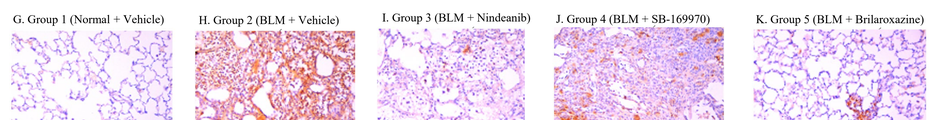
A. Group 1 (Normal + Vehicle) B. Group 2 (BLM + Vehicle) C. Group 3 (BLM + Nindeanib) D. Group 4 (BLM + SB-169970) E. Group 5 (BLM + Brilaroxazine)

F. Ashcroft Fibrosis Scores

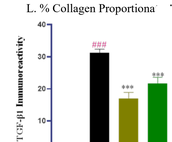


TGF β-1

G. Group 1 (Normal + Vehicle) H. Group 2 (BLM + Vehicle) I. Group 3 (BLM + Nindeanib) J. Group 4 (BLM + SB-169970) K. Group 5 (BLM + Brilaroxazine)



L. % Collagen Proportiona



Stained figures (A, F) show a significant increase in expression with COL1a1 and TGF β-1 when comparing the BLM + Veh Vs. the normal vehicle groups (1 and 2) and attenuation (B-E, F-K) with treatment. COL1a1(F) and TGF β-1 (L) immunoreactivity expression were significantly higher in BLM + Veh Vs. the normal vehicle groups (p<0.001). All treatments reflected a significant reduction in COL1a1 and TGF β-1 expression (p<0.001) Vs. the BLM + Veh group (2).