

MN-001 (*tipelukast*), a Nonselective PDE, 5-LO, LT, PLC Inhibitor

Demonstrates Anti-Fibrotic Therapeutic Effects in the Bleomycin-induced Pulmonary Fibrosis Mouse Model

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Purpose

To evaluate the effects in mice bleomycin-induced pulmonary fibrosis model, MN-001 was given orally once daily (30, 100 and 300 mg/kg) for 2 weeks. Effects of MN-001 were measured by CT evaluation of lung density, degree of pulmonary fibrosis using the Ashcroft score based on histopathological staining, and hydroxyproline content.

Background

MN-001 is a novel, orally bioavailable small molecule compound which demonstrates anti-inflammatory and anti-fibrotic activity in preclinical models. MN-001 is thought to exert phosphodiesterases (PDE) 3 and 4 inhibition, antagonism of 5-LO and LT receptor. Inhibitory effect on 5-LO and the 5-LO/LT pathway is considered to be a novel approach to treat fibrosis. MN-001 was developed for other indications and safety has been evaluated in > 600 subjects and well-tolerated with good safety profile with an open IND in the pulmonary/respiratory indication.

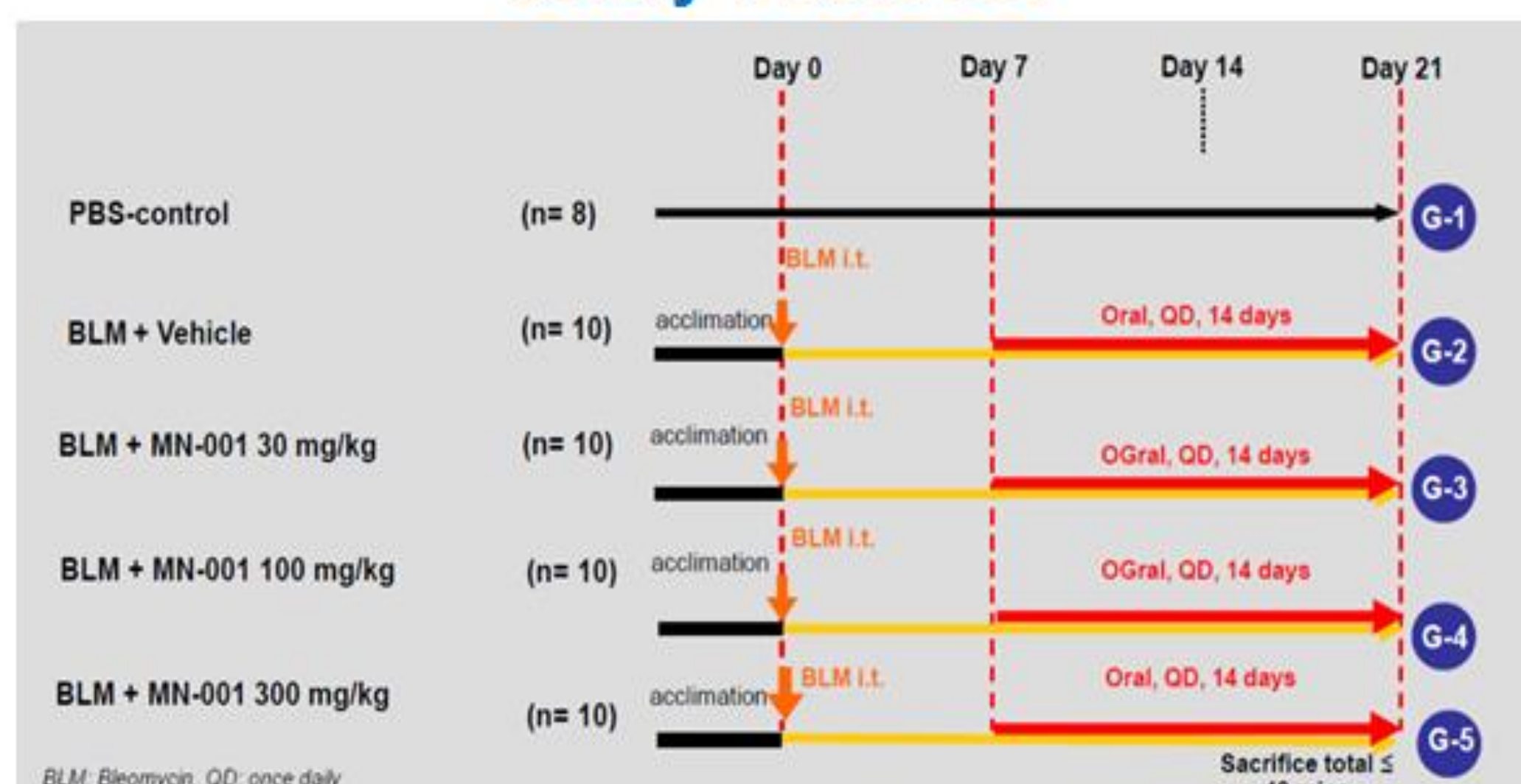
Materials & Methods

Animals: 7 week-old female C57BL/6 mice (17-21 g)

Bleomycin-Induced PF: Bleomycin (Nippon Kayaku, Japan) was given intratracheally in 50 μ L 0.9% saline per animal (N=40)

Treatment: MN-001 was given orally at doses of 30, 100, and 300 mg/kg or vehicle in a volume of 10 mL/kg once daily.

Study Protocol

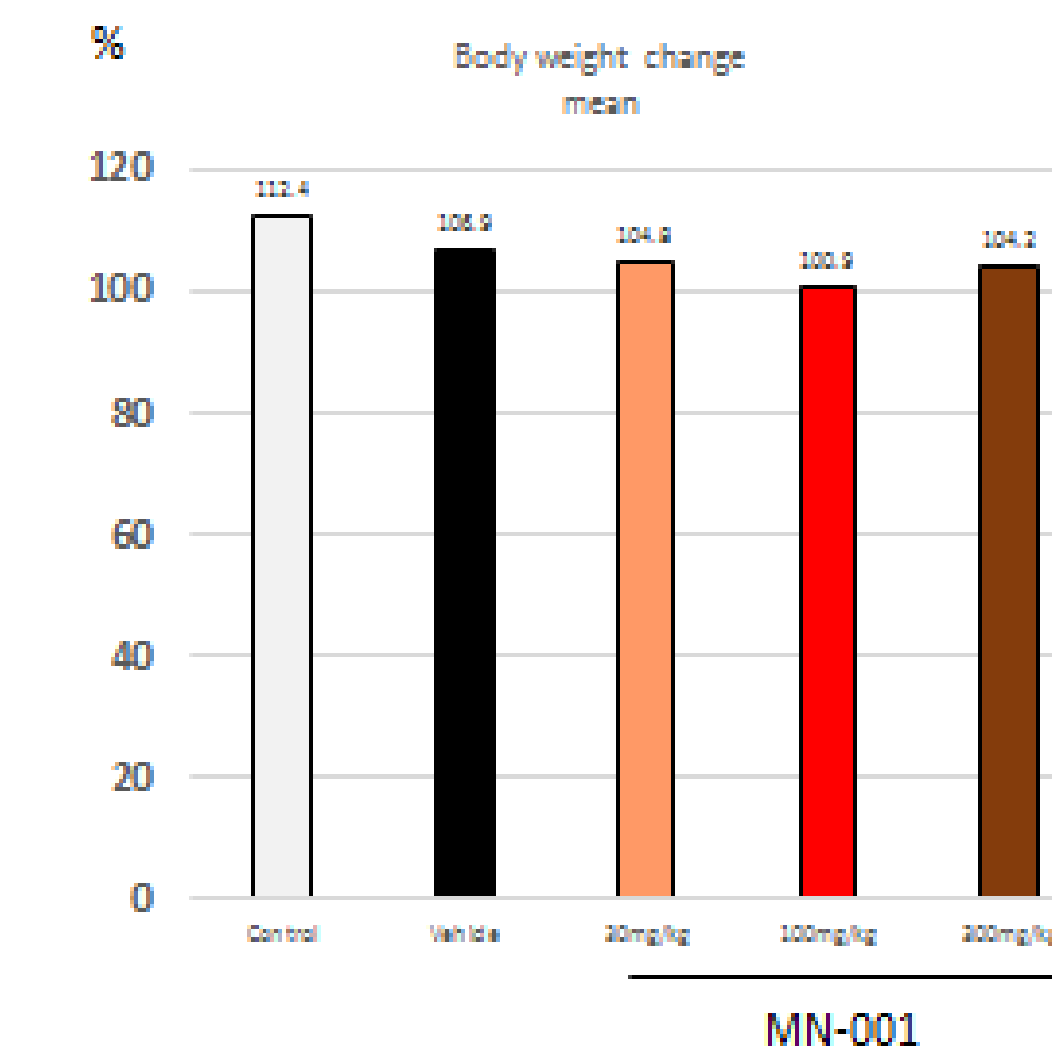


Experimental Assays

- 1) Body weight changes, general conditions, survival
- 2) CT scans to measure lung density: Mean of the of the 8 regions was defined as level of lung density
- 3) Lung hydroxyproline content : as μ g per left lung
- 4) Histopathological analyses: Ashcroft score (Masson's trichrome staining)

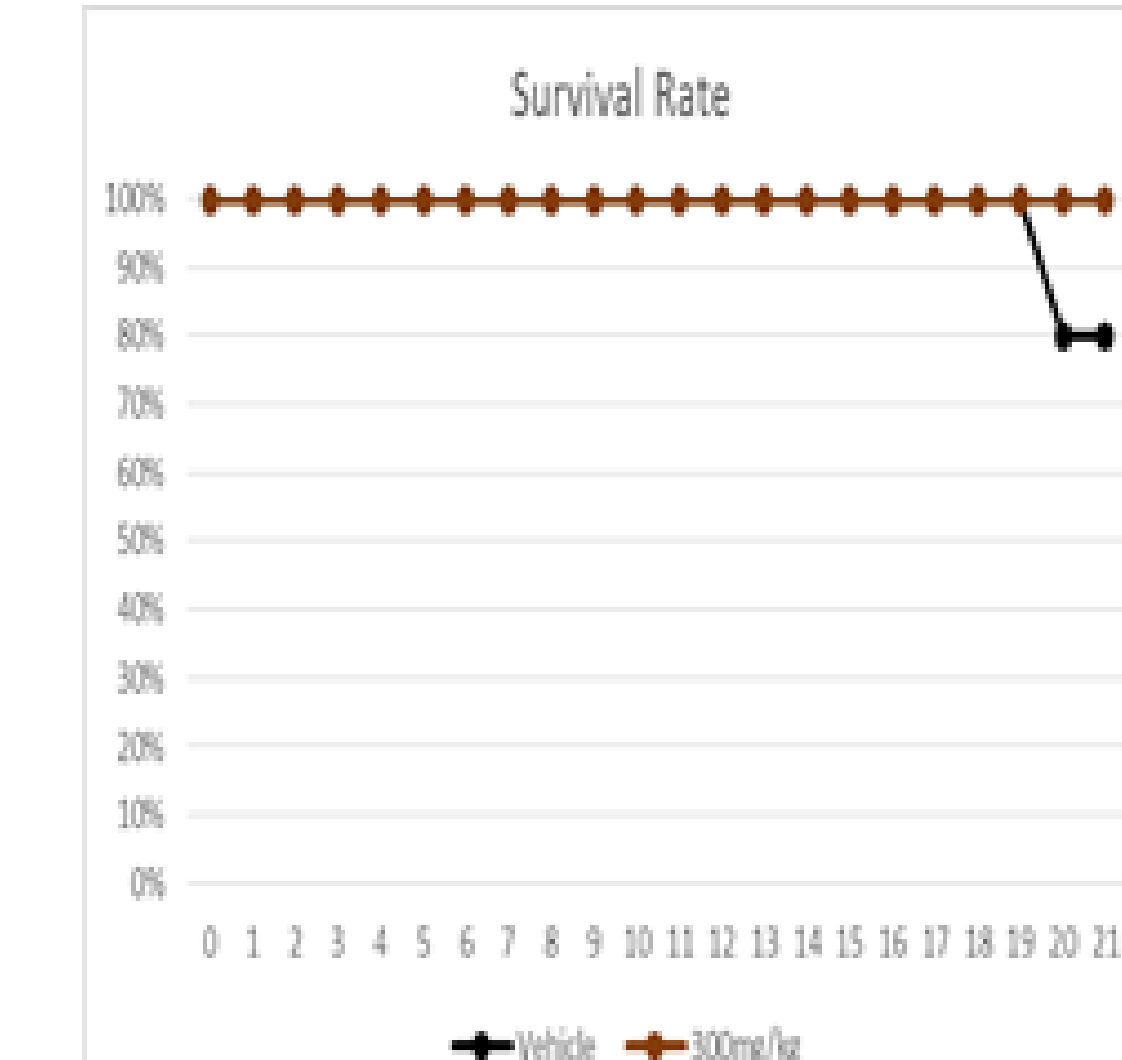
Statistical analyses were performed using Bonferroni multiple comparison tests. P values <0.05 were considered statistically significant. Results were expressed as mean \pm SD. All animals used in this study were cared for according to the Act on Welfare and Management of Animals (Ministry of the Environment, Act No. 105 of October 1, 1973); Standards Relation to the Care and Management of Laboratory Animals and Relief of Pain (Notice No. 88 of the Ministry of the Environment, April 28, 2006), and Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, June 1, 2006).

Body Weight Change Day 21 (sacrifice) from Day 0



- No significant decrease in mean body weight on day of sacrifice in Vehicle group compared with the PBS-Control group or any MN-001 treated groups

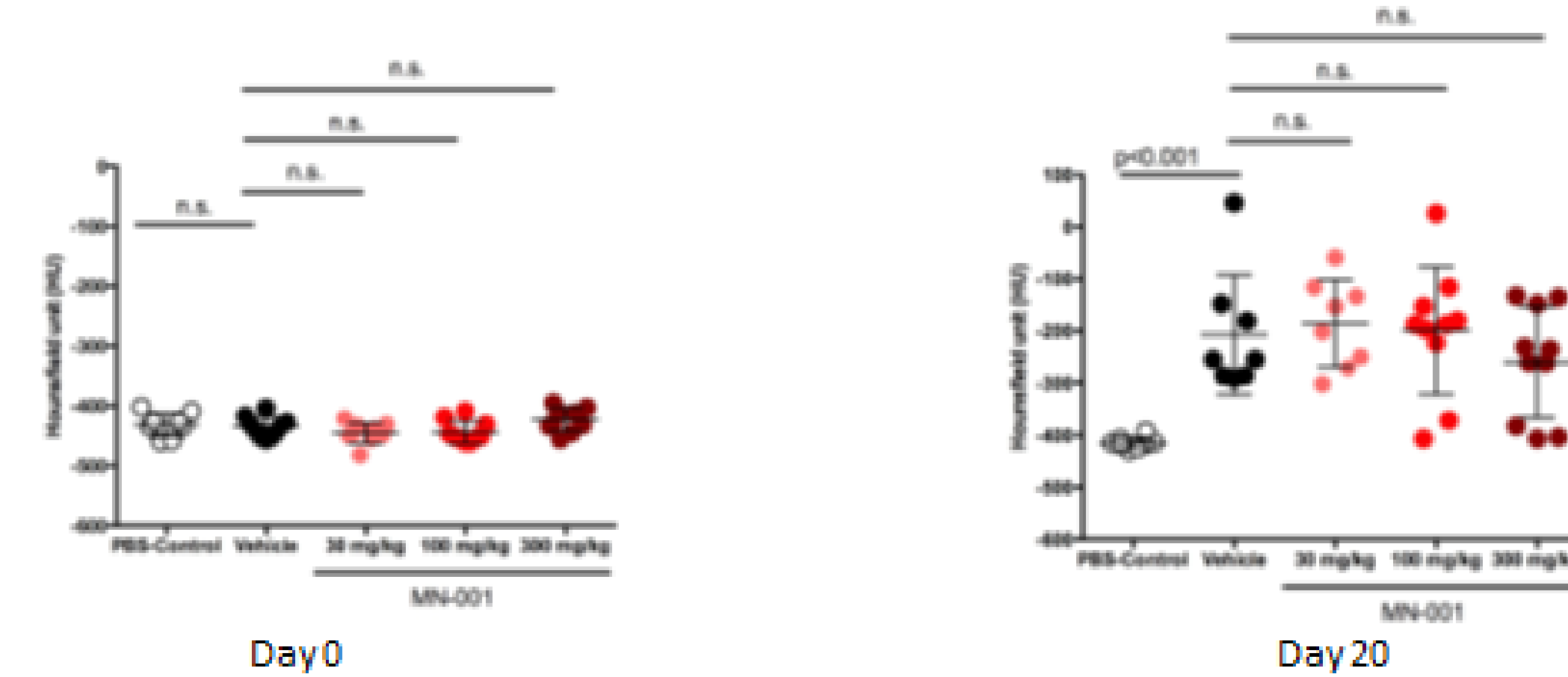
Survival Rate (%)



- During the treatment period, two out of 10 mice in the Vehicle group died before Day 21

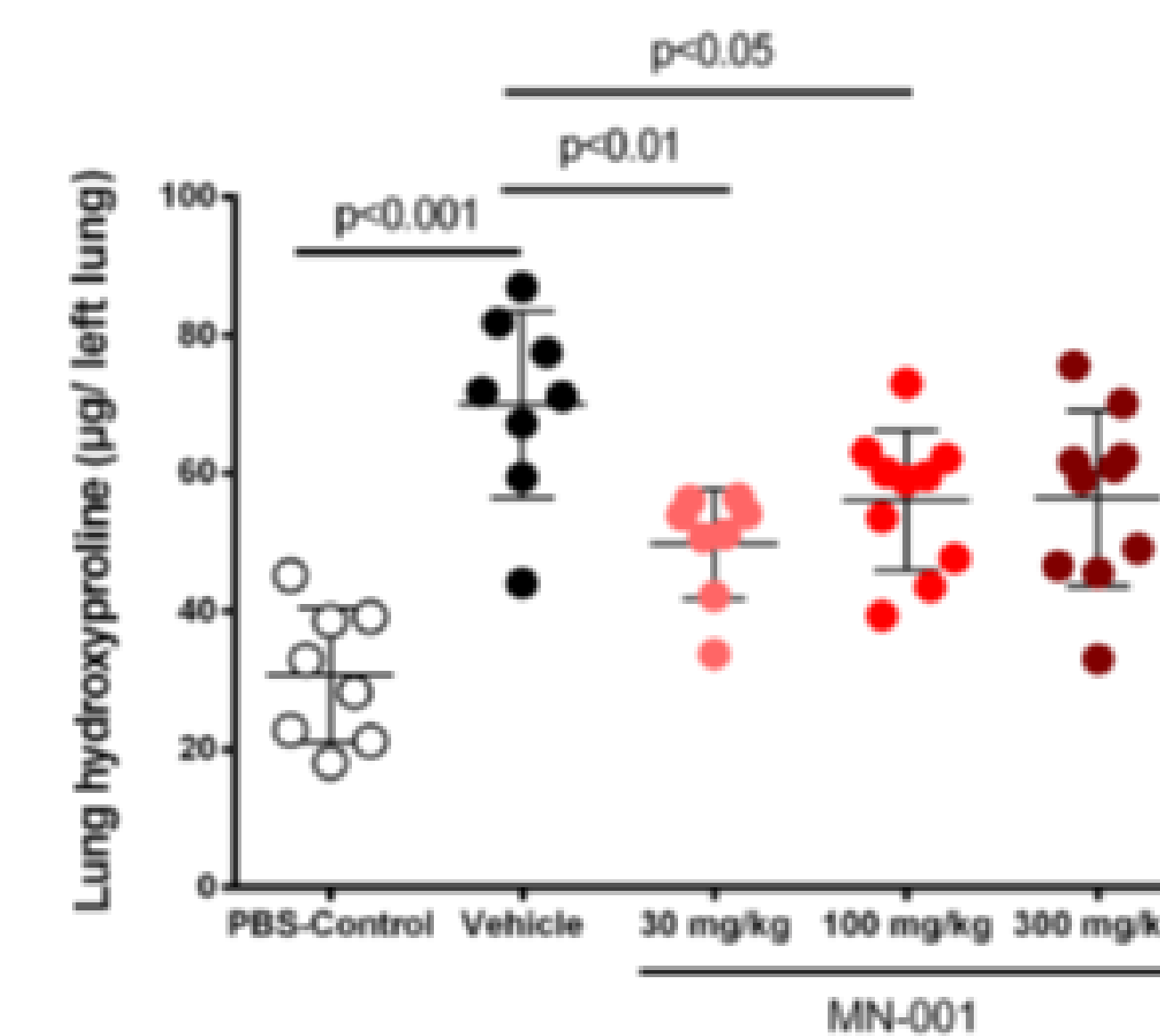
Lung density by CT scan Day 0 and 20

- Lung density measured in the Vehicle group was significantly increased on Day 20 compared to the PBS-Control group
- MN-001 tended to decrease lung density at Day 20 in a dose-dependent manner



Parameter mean \pm SD	PBS-Control (n=8)	Vehicle (n=8)	MN-001 30 mg/kg (n=8)	MN-001 100 mg/kg (n=10)	MN-001 300 mg/kg (n=10)
Lung density on day 0	-432 \pm 21	-435 \pm 17	-445 \pm 17	-444 \pm 18	-423 \pm 19
Lung density on day 21	-415 \pm 11	-207 \pm 115	-186 \pm 84	-200 \pm 122	-260 \pm 107

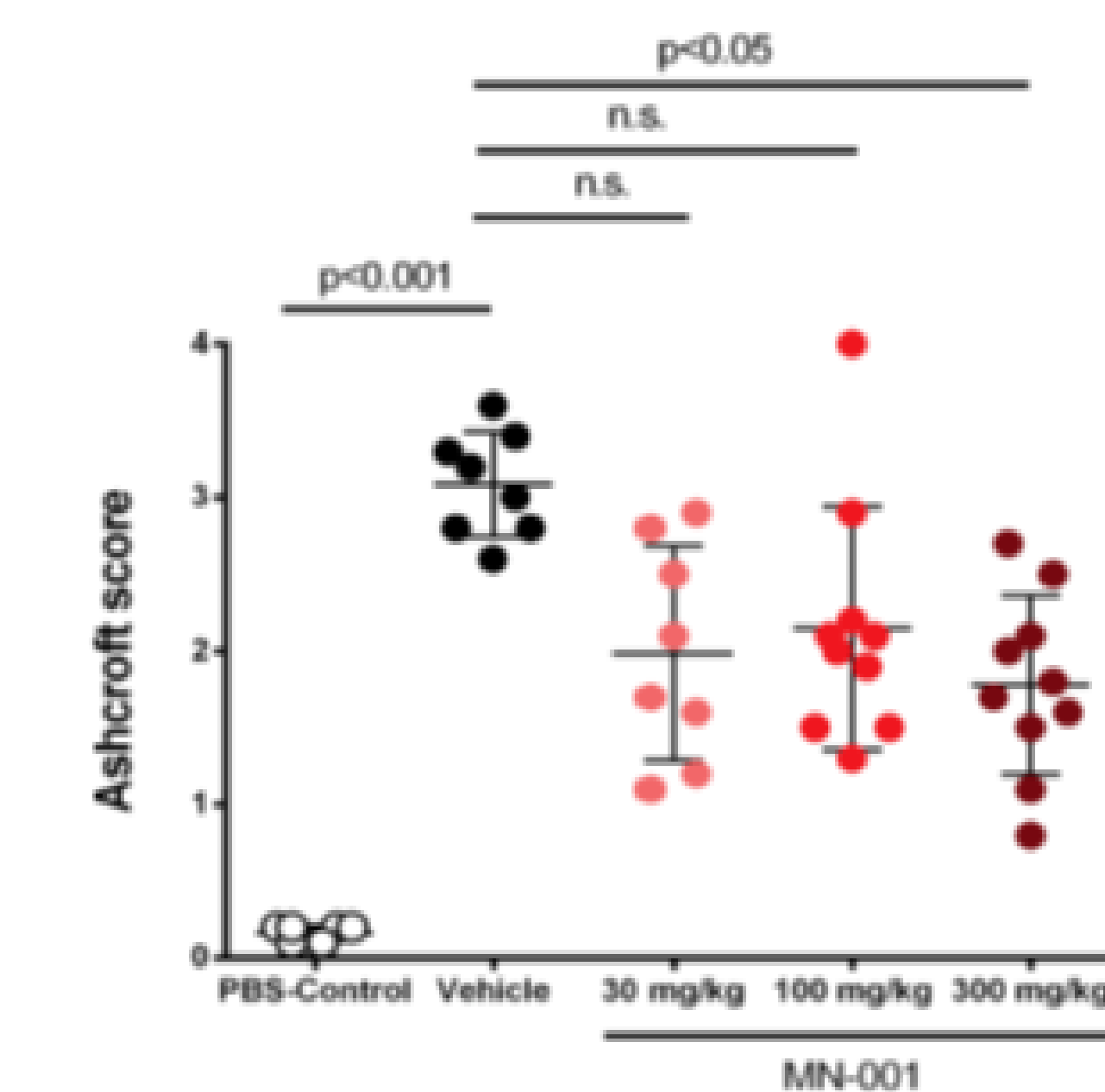
Lung Hydroxyproline Content



- Lung hydroxyproline content significantly increased in the Vehicle group compared to PBS-Control group
- In the MN-001 30 and 100 mg/kg groups, lung hydroxyproline content significantly decreased compared to Vehicle group

Parameter mean \pm SD	PBS-Control (n=8)	Vehicle (n=8)	MN-001 30 mg/kg (n=8)	MN-001 100 mg/kg (n=10)	MN-001 300 mg/kg (n=10)
Lung Hyp (μ g/ml left lung)	30.8 \pm 9.8	70.0 \pm 13.5	49.8 \pm 7.9	56.1 \pm 10.1	56.4 \pm 12.7

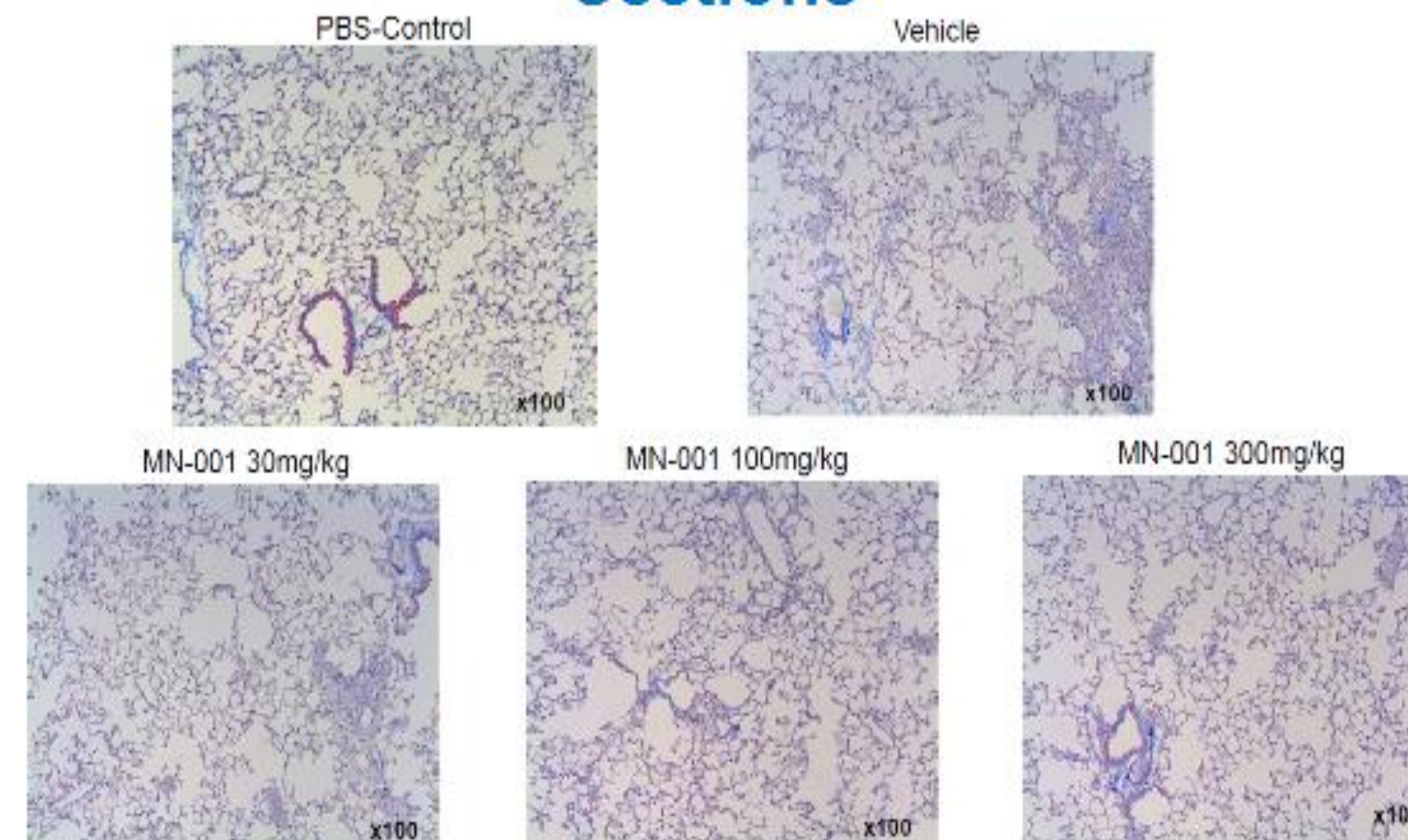
Ashcroft Score



- Significantly higher in the Vehicle group compared to PBS-Control group
- 300 mg/kg MN-001 significantly reduced Ashcroft score compared to Vehicle, which 30 and 100 mg/kg MN-001 tended to reduce Ashcroft score

Parameter mean \pm SD	PBS-Control (n=8)	Vehicle (n=8)	MN-001 30 mg/kg (n=8)	MN-001 100 mg/kg (n=10)	MN-001 300 mg/kg (n=10)
Ashcroft score	0.2 \pm 0.1	3.1 \pm 0.3	2.0 \pm 0.7	2.2 \pm 0.8	1.8 \pm 0.6

Masson's Trichrome-stained Lung Sections



Discussion and Conclusions

- Pulmonary fibrosis was established in all bleomycin-treated mice in the present study
- MN-001 showed a significant reduction or a decreasing trend in Ashcroft score, lung density, and lung hydroxyproline content compared to the Vehicle group at Day 21
- MN-001 has significant anti-fibrogenic (therapeutic) effects in the bleomycin-induced pulmonary fibrosis model in mice
- These results warrant further study to evaluate MN-001's utility in pulmonary fibrosis and other fibrotic diseases



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