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Evaluation of Prophylactic Property of PNB001, A Clinically Evaluated Anti-inflammatory New Chemical Entity in COVID-19 Patients, in Mice Induced with Lung Inflammation by Lipopolysaccharide

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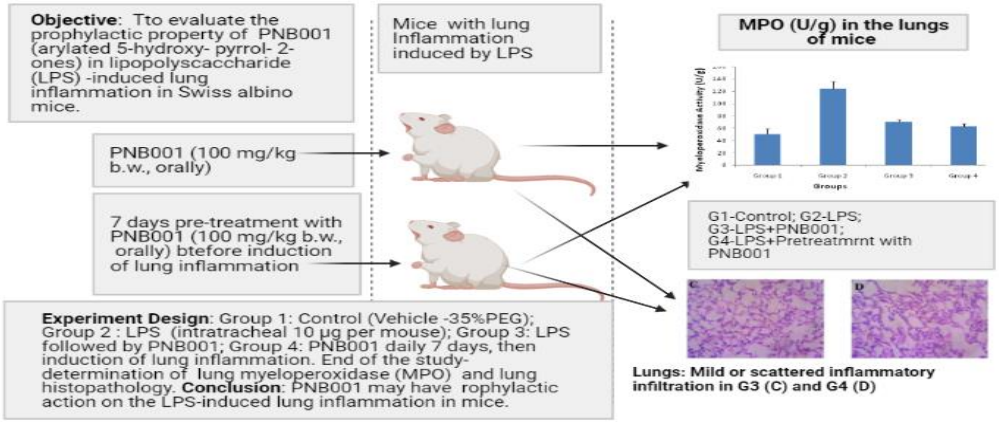
ABSTARCT

The study was conducted to evaluate prophylactic property of PNB001 (arylated 5-hydroxy-pyrrol-2-ones), a clinically evaluated anti-inflammatory and immunomodulatory new chemical entity in COVID-19 patients in Swiss albino mice. The mice were randomized into four groups (six animals/group). Group 1 which served as control received vehicle (35% polyethylene glycol (PEG) in distilled water), Group 2 received lipopolysaccharide (LPS) in saline intratracheally to induce lung inflammation (10 µg per mouse), and Group 3 received LPS as in Group 2 followed by PNB001 orally at 100 mg/kg b.w. Group 4 received daily oral administration of PNB001 at 100 mg/kg b.w. for 7 days, then lung inflammation was induced in them as in Groups 2 and 3. Myeloperoxidase (MPO) activity was determined in all groups of animals at 30 h following the induction of lung inflammation by LPS. MPO significantly increased in Group 2 compared to Group 1, and decreased significantly in Groups 3 and 4 compared to Group 2. Histopathological examination of the lungs revealed severe infiltration of inflammatory cells in Group 2, the severity of which was to a lesser degree in Groups 3 and 4. It is concluded from the study that oral administration of PNB001 at 100 mg/kg b.w. has decreased the LPS-induced lung inflammation in mice. Daily oral administration of PNB001 at 100 mg/kg b.w. for 7 days has prophylactic action on the LPS-induced lung inflammation in mice. PNB001 has an immunomodulatory effect and may have the potential to be considered as a pre-exposure prophylaxis drug against inflammation caused by COVID-19.



Graphical Abstract

KEY WORDS: PNB001, Cholecystokinin, CCK, Anti-inflammatory, MPO, Myeloperoxidase, Prophylaxis, COVID-19.

1. INTRODUCTION

Respiratory distress caused by alveolitis is the serious effect of the COVID-19 infection (Gallelli, 2020; Pandolfi, 2020). During COVID-19 infection, a large amount of pro-inflammatory cytokines gets released resulting in an aggressive inflammatory response in the lungs. COVID-19 infection-caused alveolitis was first detected in Wuhan (China) in December 2019 (Wu, 2020). Alveolitis results in the accumulation of protein-rich fluid in the lungs causing impairment of arterial oxygenation. Hence, supplemental oxygen is the most important vital step for the treatment of severe COVID-19 patients (Dondorp, 2020). Recently, several countries have seriously focused their attention on improving the capacity to supply oxygen in hospitals (Bikkina, 2021).

PNB001, a new chemical entity, is a first-in-class cholecystokinin-A (CCK-A) agonist and CCK-B antagonist, a unique anti-inflammatory agent with immune stimulation properties. It has shown analgesic, antipyretic and anti-inflammatory properties in animal models (Lattmann, 2017). It acts on the inflammatory cytokines by the cholinergic anti-inflammatory pathway and the gastrin-releasing peptide receptor pathway. The cholecystokinin via the CCK-2 pathway reduces inflammation in addition to the CCK-1 cholinergic anti-inflammatory pathway.

A clinical trial conducted in moderate COVID-19 patients indicated that PNB001 at a dose of 100 mg three times a day for 14 days has marked beneficial effects in the patients. The clinical trial also showed that PNB001 possesses an immunomodulatory effect (Lattmann, 2021). The present study is designed to evaluate the prophylactic property of PNB001 in LPS-induced lung inflammation in Swiss albino mice.

2. METHODS

PNB001: PNB001 (arylated 5-hydroxy-pyrrol-2-ones), which can be prepared in 2 synthetic steps from mucochloric acid (Lattmann, 2017) is a highly potent and selective gastrin antagonist with anti-inflammatory and analgesic properties (Lattmann, 2018). Clinical phase 1 study conducted in 74 healthy subjects and phase 2 study conducted in 42 subjects revealed that PNB001 is safe and with an efficient therapeutic window (Lattmann, 2021).

Animal ethics: The study protocol was approved by Institute's Animal Ethics Committee. This study was performed as per the recommendations and ethical practices as laid down in the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India for animal care. The number of animals used in this study was considered to be the minimum requirement to meet rational scientific endpoints.

Female albino mice (2 months old, 23-28 g body weight) procured from Biogen Animal facility, Hosur, were acclimated to laboratory environment (12 h light/12 h dark cycle, 40-60% relative humidity and 20-24ºC) for 15 days in the experimental room. During the acclimation and experimental period, the animals were fed with a commercial pellet diet (Sai Animal Feed Ltd, Chennai), and water was given ad libitum. Following acclimation, the mice were randomly divided into four groups (six animals per group). Lung inflammation was induced by LPS instillation (intratracheal at 10 µg per mouse) in the mice belonging to groups 2 and 3. Group 1 served as vehicle control, which received only vehicle (35% PEG in distilled water) orally at 1 and 6 h on day 1, whereas Group 3 received PNB001 (suspended in 35% PEG in distilled water) orally at 100 mg/kg body weight 1 h before and 6 h after LPS instillation on day 1. Group 4 received daily oral administrations of PNB001 at 100 mg/kg body weight for 7 days, then lung inflammation was induced in these animals by intratracheal instillation with LPS (10 µg per mouse), and observed for another day without any treatment. The volume of vehicle administered in Group 1 and PNB001 in Groups 3 and 4 was 10 ml/kg body weight. The mice belonging to Groups 2, 3, and 4 were observed for 30 h without any treatment following the LPS instillation. Then these animals and those belonging to the control group were sacrificed for the separation of lungs for the determination of MPO activity and histopathology.

Intratracheal instillation of LPS: Mice, under ketamine–xylazine (50-10 mg/kg body weight, intraperitoneal administration) anesthesia, were instilled intratracheally with 50 µl saline containing LPS (10 µg per mouse) (LPS-Escherichia coli 055:B5 was obtained from Sigma India, Bangalore), after blunt dissection of the neck soft tissues to expose the trachea.

D**etermination of MPO activity in the lungs:** The animals were killed under ether deep anesthesia. The lung was then removed to estimate MPO activity (Xie, 2009) and for histopathological examination.

Histopathological examination: The right lungs were aseptically removed, inflated, and then fixed with 10% paraformaldehyde; the tissue samples were embedded in paraffin, sectioned at 5 µ thickness, and stained with hematoxylin and eosin (H&E).

Statistical analysis: MPO data were analyzed using one-way ANOVA. Post-hoc comparisons among the groups were done using Student-Newman-Keuls' test. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

MPO activity significantly increased in animals induced with lung inflammation by tracheal LPS instillation (Group 2). In the mice, on treating with PNB001 at 100 mg/kg body weight 1 h before and 6 h after tracheal LPS instillation (Group 3) or daily administrations of PNB001 at 100 mg/kg body weight for 7 days before induction of lung inflammation (Group 4), MPO activity significantly decreased in the lungs compared to Group 2 (Figure.1).

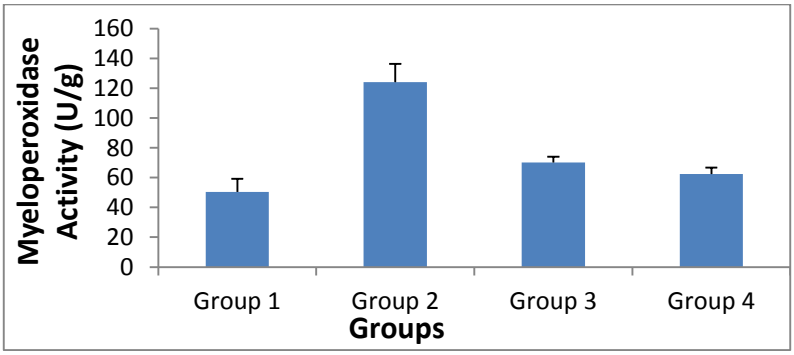


Figure.1. MPO (U/g) in the lungs of mice induced with lung inflammation by LPS

Each histogram represents the mean value, and the error bar represents the standard deviation of each mean value (mean ± SD, n = 6). Group 2 is different from all the other Groups ($P<0.05$); Groups 3 and 4 are similar ($P>0.05$), but different from Group 1 ($P<0.05$) (Student-Newman-Keul's test).

Coronavirus disease 2019 (COVID-19), has become a pandemic (Wang, 2020). This disease has caused a serious impact on the economy of several countries. Over a million people across the world died due to respiratory distress associated with COVID-19 or related complications (WHO, 2020). The second wave of this dreadful disease is more severe than the first one as the viruses are with a different genetic composition and spread faster than the previous wave. Though several studies have been carried out on the clinical management of COVID-19, the efficacy of most drugs used for treating COVID-19 patients is not clinically validated. For example, the interim results of the Solidarity Trial indicated that the four drugs widely used across the world such as Remdesivir, hydroxychloroquine, lopinavir/ritonavir, and interferon had very little or no effect on overall mortality, initiation of ventilation, and duration of hospital stay in hospitalized patients (WHO, 2021). One of the reasons for the ineffectiveness of the drugs currently used for respiratory distress for treating COVID-19 patients is the unique pathophysiology of COVID-19 (Yang, 2020). In severe COVID-19 cases, the immune response increases tremendously, causing a "cytokine storm". This "cytokine storm" results in the formation of reactive oxygen species (ROS) (Liu, 2016). The infiltrating neutrophils release MPO, which activates several pathways that lead to elevated cytokines and the production of ROS (Abu-Soud, 2014). MPO contributes significantly to the vascular damage in alveoli in COVID-19 patients, as MPO causes a decrease in nitric oxide, consequently leading to vasoconstriction (Goud, 2021). Several authors have reported the release of MPO during extensive inflammation (Haegens, 2008) and it is considered as a marker of inflammation (Loria, 2008). In the present study, MPO markedly increased in the lungs of mice treated with LPS indicating severe lung inflammation. The decrease in MPO in the PNB001 treated Group 3 indicates the curative action of the drug and in the Group 4 indicates that the drug may have prophylactic property. The curative and prophylactic activity of PNB001 is further confirmed in the histopathology of the lings (Figure.2).

LPS generates severe inflammation in mice on intratracheal administration, showing increased vascular permeability, interstitial and alveolar edema, and a flood of flowing inflammatory cells (Chen, 2010). In the present study, intratracheal administration of LPS resulted in a flow of inflammatory infiltrates (lymphocytes and neutrophils) in the lungs (Figure.2B). Such infiltration of the cells is reported in COVID-19 patients (Deshmukh, 2021). In the mice treated with PNB001 (Group 3), the severity of the inflammation decreased markedly (Figure.2C). Similarly in the mice treated with PNB001 before the induction of lung inflammation showed a decrease in the severity of lung inflammation (Figure.2D). PNB001 administered groups also showed less inflammatory filtrates compared to Group 2. The decrease in inflammatory filtrates observed in Group 4 indicates that PNB001 possesses prophylactic property against lung inflammation induced by LPS. The immunomodulatory property of PNB001 was observed in Phase II clinical trial conducted in COVID-19 patients (Lattmann, 2021).

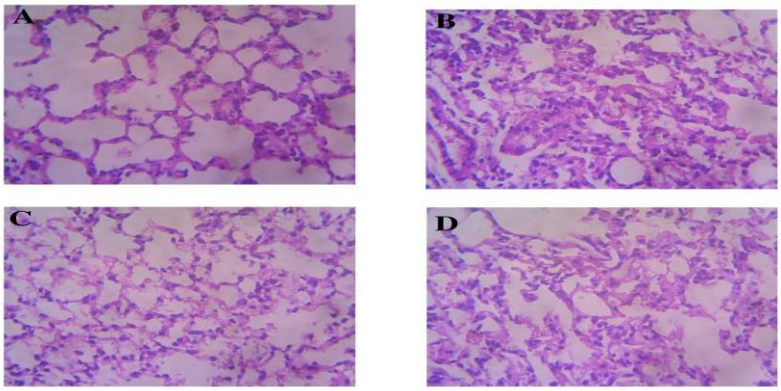


Figure.2. Histopthology of the lung of mice, H&E staining (40X magnification)

Group 1 (Fig.2A): Lung showing normal alveoli. Interstitium shows thin walled congested vessels. Bronchi and bronchioles show no significant pathology.

Group 2 (Fig.2B): Lung showing irregular dilatation of alveoli, with few area showing compressed alveoli. Interstitium shows thin walled congested vessels with inflammatory infiltrates composed of lymphocytes and neutrophils. Blood vessels show congestion.

Group 3 (Fig.2C): Showing mild inflammatory infiltration.

Group 4 (Fig.2D): Showing normal alveoli. Interstitium shows scattered infiltration of lymphocytes, macrophages. Bronchi and peribronchial region show normal. Blood vessels show no significant pathology**.**

4. CONCLUSION

Oral administration of PNB001 at 100 mg/kg b.w. has decreased the severity of the LPS-induced lung inflammation in mice. Daily oral administration PNB001 at 100 mg/kg b.w. for 7 days has prophylactic action on the LPS-induced lung inflammation in mice. PNB001 may have an immunomodulatory effect and has the potential to be considered as a pre-exposure prophylaxis drug against COVID-19.

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