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[Ga-Young Lee](#) , Won Se Lee , Jisung Han , [Yung-Choon Yoo](#) *

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Article

Oral and Intranasal Administration of Polydeoxyribonucleotide Isolated from *Porphyra* sp. Ameliorates Acute Lung Injury via Suppressing Proinflammatory Cytokine Production in Mice

Ga-Young Lee ¹, Won Se Lee ^{2,3}, Jisung Han ^{2,3} and Yung-Choon Yoo ^{1,*}

¹ Department of Microbiology, College of Medicine, Konyang University, Daejeon 32992, Republic of Korea

² All In One GENETECH Ltd., Busan 46508, Republic of Korea

³ DERMARE Co. Ltd., Busan 48480, Republic of Korea

* Correspondence: yc_yoo@konyang.ac.kr; Tel.: +82-42-600-8694

Abstract

Acute lung injury (ALI) is a severe inflammatory condition with high mortality rates, necessitating the development of effective therapeutic agents. Polydeoxyribonucleotide (PDRN), a DNA-derived compound known for its tissue repair and anti-inflammatory properties, has gained attention as a potential therapeutic agent. However, the efficacy of PDRN derived from marine sources, particularly *Porphyra* sp. (laver), remains unexplored in respiratory inflammation. In this study, we investigated the protective effects of *Porphyra* sp.-derived PDRN (Ps-PDRN) against LPS-induced ALI in mice through two administration routes: intranasal (IN) and oral (PO). Ps-PDRN treatment significantly attenuated fever, pulmonary edema, and histopathological changes in LPS-challenged mice. Both IN and PO administration of Ps-PDRN markedly reduced pro-inflammatory cytokines (TNF- α , IL-1 β , IL-6) and chemokines (MCP-1, RANTES, CXCL1, MIP-2) in bronchoalveolar lavage fluid (BALF) and serum. Comparative analysis of the two administration routes revealed distinct efficacy profiles, with oral administration demonstrating superior chemokine inhibition while intranasal delivery showed advantages in certain cytokine suppression. Histological examination revealed that Ps-PDRN preserved alveolar architecture and reduced inflammatory cell infiltration. Furthermore, *in vitro* studies using RAW 264.7 macrophages demonstrated that Ps-PDRN inhibited LPS-induced production of proinflammatory cytokines such as TNF- α and IL-6 in a dose-dependent manner. These findings suggest that Ps-PDRN exerts potent anti-inflammatory effects against ALI through both local and systemic administration routes, highlighting its potential as a novel therapeutic agent for inflammatory lung diseases.

Keywords: *Porphyra* sp.; polydeoxyribonucleotide; PDRN; acute lung injury; inflammation; cytokines; chemokines; administration route

1. Introduction

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are life-threatening inflammatory conditions characterized by diffuse alveolar damage, pulmonary edema, and respiratory failure [1]. Despite advances in critical care medicine, ALI remains associated with significant morbidity and mortality, with limited therapeutic options available [2]. The pathogenesis of ALI involves complex inflammatory cascades triggered by various insults, including bacterial infections, where lipopolysaccharide (LPS) from gram-negative bacteria plays a pivotal role in initiating and amplifying the inflammatory response [3].

LPS activates alveolar macrophages and epithelial cells by binding to its receptor toll-like receptor 4 (TLR4) on the cells, and triggers the release of pro-inflammatory cytokines such as tumor

necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6), as well as chemokines including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2) [4,5]. These inflammatory mediators promote neutrophil recruitment, increase vascular permeability, and cause tissue damage, ultimately leading to impaired gas exchange and respiratory failure [6]. Therefore, therapeutic strategies targeting excessive inflammatory responses represent a promising approach for ALI management. Polydeoxyribonucleotide (PDRN) is a mixture of deoxyribonucleotide polymers included in various biological sources, typically salmon sperm [7]. PDRN has been extensively studied for its tissue repair, wound healing, and anti-inflammatory properties, primarily through activation of adenosine A2A receptors [8,9]. Clinical applications of PDRN include treatment of chronic wounds, osteoarthritis, and various inflammatory conditions [10,11]. However, most studies have focused on PDRN derived from salmon (*Oncorhynchus* spp.), and the therapeutic potential of PDRN from alternative marine sources remains largely unexplored.

Porphyra sp., commonly known as laver or nori, is a marine red algae widely consumed as a traditional food in East Asian countries [12]. This edible seaweed is rich in bioactive compounds including polysaccharides, proteins, and nucleic acids [13]. Recent advances in extraction technology have enabled the isolation of PDRN from *Porphyra* sp., yielding a mixture of polydeoxyribonucleotide and polynucleotide with a molecular weight range of 5–1,000 kDa [14]. Given the abundant availability and sustainable cultivation of *Porphyra* sp., this marine-derived PDRN represents a promising alternative source for therapeutic applications. In recent, we demonstrated that *Porphyra* sp.-derived PDRN (Ps-PDRN) has anti-inflammatory activity to inhibit nitric oxide (NO) production from LPS-stimulated RAW 264.7 macrophages by suppressing the activation of mitogen-activated protein kinase (MAPK) such as extracellular signal-regulated kinase (ERK) and p38 [15].

In this study, we investigated the inhibitory effects of Ps-PDRN on ALI in an *in vivo* mouse model, and analyzed the related immunological mechanisms in lung tissue. Notably, we compared two administration routes—intranasal (IN) and oral (PO)—to evaluate both local and systemic therapeutic approaches. Additionally, we examined the *in vitro* anti-inflammatory activity of Ps-PDRN using LPS-stimulated RAW 264.7 macrophages. Our findings provide the first evidence for the protective effects of *Porphyra* sp.-derived PDRN against ALI, suggesting its potential as a novel therapeutic agent for inflammatory lung diseases.

2. Materials and Methods

2.1. Preparation of Ps-PDRN

Ps-PDRN were prepared according to the method previously described [15]. Briefly, *Porphyra* sp. was washed three times with tap water to remove surface epiphytes, residual salts, and sand, followed by careful rinsing with fresh water. The cleaned samples were stored at -20°C until processing, then freeze-dried and finely homogenized to obtain a dry powder. The powdered *Porphyra* sp. was lysed in a protein lysis buffer composed of 10 wt% soybean, 10 wt% adlay, 20 wt% green tea extract, 20 wt% soybean fatty acid, 20 wt% tocopherol, cocamidopropyl betaine, and 20 wt% olive oil carboxylate. Ribonucleases were subsequently added to separate polynucleotides and PDRN. The lysate was then subjected to centrifugation and filtration to remove insoluble residues and other impurities. The clarified filtrate was dried, washed, and centrifuged to obtain low-molecular weight Ps-PDRN. The final extract was concentrated by evaporation and lyophilized, yielding powdered Ps-PDRN, which was stored at -80°C until use. The purity of Ps-PDRN was assessed by measuring the absorbance ratio at 260 and 280 nm (A_{260}/A_{280}), and the DNA concentration was determined from the absorbance at 260 nm using a microplate reader (PowerWave XS2, BioTek Instruments, Inc.). The purity of isolated Ps-PDRN was $\geq 99\%$.

2.2. Cell Culture and Cytotoxicity Assay

RAW 264.7 murine macrophage cells were obtained from the Korean Cell Line Bank (Seoul, Republic of Korea) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented

with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a humidified atmosphere with 5% CO₂. Cytotoxicity of Ps-PDRN was assessed using the MTT assay. Cells (5 × 10⁴/well) were seeded in 96-well plates and incubated for 8 h. Cells were then treated with various concentrations of Ps-PDRN (0–100 µg/mL) for 12 h, followed by stimulation with LPS (1 µg/mL; *Escherichia coli* serotype 055:B5, Sigma-Aldrich, St. Louis, MO, USA) for 24 h. MTT solution (5 mg/mL) was added, and cells were incubated for 2 h. After centrifugation, the supernatant was removed, and formazan crystals were dissolved in dimethyl sulfoxide. Absorbance was measured at 540 nm using a microplate reader.

2.3. Determination of Cytokines in Cell Culture

RAW 264.7 cells were pretreated with Ps-PDRN at indicated concentrations for 12 h, followed by LPS (100 ng/mL) stimulation for 24 h. The levels of TNF-α and IL-6 in the culture supernatants were quantified using enzyme-linked immunosorbent assay (ELISA) kits (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions.

2.4. Real-Time PCR Analysis

Total RNA was extracted from RAW 264.7 cells or lung tissues using TRIzol reagent (iNtRON Biotechnology, Seongnam, Republic of Korea). cDNA was synthesized using a power cDNA synthesis kit (iNtRON Biotechnology), and quantitative real-time PCR was performed using Cfx96 (Bio-Rad, Hercules, CA, USA). The expression levels of TNF-α, IL-1β, and IL-6 were normalized to GAPDH. The primer sequences are listed in Table 1.

Table 1. Primer sequences for quantitative real-time PCR analysis.

Gene	Primer Sequence (5'→3')	Accession No.	Catalog No.
TNF-α	F: GGTGCCTATGTCTCAGCCTCTT R: GCCATAGAACTGATGAGAGGGAG	NM_013693	MP217748
	F: TGGACCTTCCAGGATGAGGACA R: GTTCATCTCGGAGCCTGTAGTG	NM_008361	MP206724
IL-6	F: TACCACTTCACAAGTCGGAGGC R: CTGCAAGTGCATCATCGTTGTTC	NM_031168	MP206798
	F: CATCACTGCCACCCAGAAGACTG R: ATGCCAGTGAGCTTCCCGTTTCAG	NM_008084	MP205604

All primers were obtained from OriGene Technologies, Inc. (Rockville, MD, USA). F, forward primer; R, reverse primer.

2.5. Animal Experiments

Seven-week-old male BALB/c mice (19–22 g) were purchased from Raon Bio (Yongin, Republic of Korea). All animal experiments were conducted in accordance with the guidelines approved by the Animal Ethics Committee of Konyang University (Approval No. P-25-12-A-01). Mice were randomly divided into seven groups (n = 7 per group): (1) Normal control; (2) LPS (vehicle); (3) LPS + Dexamethasone (DEX, 5 mg/kg/mouse, positive control); (4) LPS + Ps-PDRN IN-Low (IN-L, 25 µg/mouse); (5) LPS + Ps-PDRN IN-High (IN-H, 50 µg/mouse); (6) LPS + Ps-PDRN PO-Low (PO-L, 100 µg/mouse); and (7) LPS + Ps-PDRN PO-High (PO-H, 200 µg/mouse). For intranasal administration groups, Ps-PDRN was administered intranasally once daily for three days before ALI induction. For oral administration groups, Ps-PDRN was administered by oral gavage once daily for three days before ALI induction. ALI was induced by intranasal administration of LPS (100 µg/mouse). Mice were sacrificed 18 h after LPS administration, and lung tissues, blood, and bronchoalveolar lavage fluid (BALF) were collected for analysis.

2.6. Body Temperature and Lung Edema Assessment

Body temperature was measured using a rectal thermometer 24 h after LPS administration. For lung edema assessment, lungs were excised, weighed (wet weight), and dried in an oven at 60 °C for 48 h to obtain dry weight. The wet/dry (W/D) ratio was calculated as an indicator of pulmonary edema.

2.7. BALF and Serum Analysis

BALF was collected by lavaging the lungs three times with 1 mL of cold PBS. Blood was collected by cardiac puncture and centrifuged to obtain serum. The concentrations of cytokines (TNF-α, IL-1β, IL-6) and chemokines (MCP-1, RANTES, CXCL1, MIP-2) in BALF and serum were measured using ELISA kits (BD Biosciences) according to the manufacturer's protocols.

2.8. Histological Analysis

Lung tissues were fixed in 4% paraformaldehyde (Image-IT™ Fixative Solutions; Invitrogen, Waltham, MA, USA), dehydrated with 30% sucrose solution, and embedded in Tissue-Tek OCT compound (Sakura Finetek USA Inc., Torrance, CA, USA). Frozen sections were prepared using a cryosectioning machine (CM1520; Leica Biosystems, Nussloch, Germany) and stained with hematoxylin and eosin (H&E) for histopathological examination. Additionally, BALF cells were cytopun onto glass slides and stained with Diff-Quik for morphological analysis of alveolar macrophages.

2.9. Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics 25 and SAS 9.4. Differences between groups were analyzed using Student's *t*-test. *p* < 0.05 was considered statistically significant.

For the comparative analysis of administration routes, percent inhibition rates were calculated as [(LPS – Treatment) / (LPS – Normal)] × 100 to quantify the relative efficacy of each treatment in reversing LPS-induced changes. Effect sizes were calculated using Cohen's *d* with 95% confidence intervals to assess the magnitude of treatment effects compared to the LPS control group, where |*d*| = 0.2, 0.5, and 0.8 represent small, medium, and large effects, respectively.

Supplementary statistical analyses and data visualizations were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA) and Orange 3 (University of Ljubljana, Slovenia). Heatmap visualization (Figure S1) was generated to display inhibition rates across all markers and treatment groups. Radar charts (Figure S2) were constructed to compare efficacy profiles between intranasal and oral administration routes. Forest plots (Figure S3) were created to present effect sizes with 95% confidence intervals. Comparative bar charts (Figure S4) and dose-response curves (Figure S5) were

generated to illustrate route-specific patterns and dose-dependent effects. Literature review data (Table S1) were compiled from PubMed searches using the terms "PDRN," "polydeoxyribonucleotide," and "anti-inflammatory" to contextualize the current findings within existing PDRN research.

3. Results

3.1. Ps-PDRN Attenuates LPS-Induced Fever and Pulmonary Edema

Intranasal administration of LPS induced significant increases in body temperature (from 35.8 °C to 38.0 °C) and lung wet/dry ratio in mice, indicating the successful establishment of ALI model (Figure 1). Treatment with Ps-PDRN via both IN and PO routes significantly reduced the LPS-induced elevation in body temperature. The IN-L (25 µg/mouse) and IN-H (50 µg/mouse) groups showed body temperatures comparable to the dexamethasone-treated group. Interestingly, both PO-L (100 µg/mouse) and PO-H (200 µg/mouse) groups showed significant antipyretic effects. Pulmonary edema, assessed by the lung W/D ratio, was markedly increased following LPS challenge. Both IN and PO administration of Ps-PDRN significantly reduced the W/D ratio in a dose-dependent manner, with the PO-L group showing the most pronounced effect (Figure 1B). Comparative analysis revealed that oral administration achieved 56.3% inhibition of pulmonary edema compared to 39.8% for intranasal administration (Supplementary Figure S1). These results indicate that Ps-PDRN effectively ameliorates systemic inflammation and pulmonary edema associated with ALI.

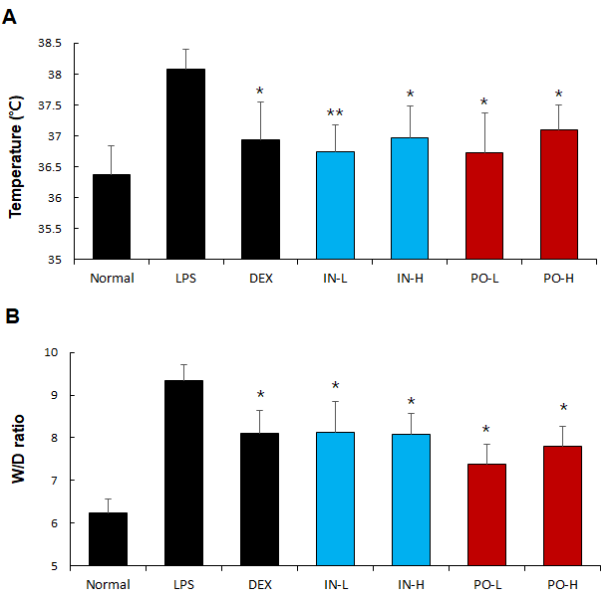


Figure 1. Ps-PDRN attenuates LPS-induced fever and pulmonary edema. (A) Body temperature; (B) Lung wet/dry (W/D) ratio. Data are presented as mean ± SD (n = 7). * $p < 0.05$, ** $p < 0.01$ vs. LPS group.

3.2. Ps-PDRN Reduces Pro-Inflammatory Cytokines in BALF

LPS administration markedly elevated the concentrations of pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6 in BALF (Figure 2). Treatment with Ps-PDRN via both routes significantly suppressed these cytokine levels. For TNF- α , all treatment groups showed significant reductions compared to the LPS group, with the IN-H group demonstrating the most potent inhibition (Figure 2A). IL-1 β levels were significantly reduced in all Ps-PDRN-treated groups, with PO-L showing comparable efficacy to dexamethasone (Figure 2B). IL-6 concentrations were also markedly decreased in both IN and PO groups, with intranasal administration showing superior efficacy (83.3% inhibition) compared to oral administration (59.7% inhibition) (Figure 2C; Supplementary Figure S2).

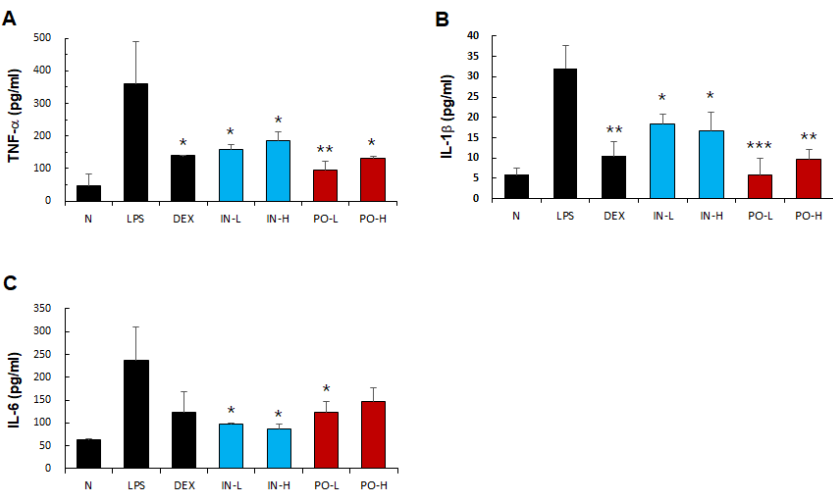


Figure 2. Effects of Ps-PDRN on pro-inflammatory cytokine levels in BALF. (A) TNF-α; (B) IL-1β; (C) IL-6. Data are presented as mean ± SD (n = 7). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. LPS group.

3.3. Ps-PDRN Reduces Cytokine mRNA Expression in Lung Tissues

To examine the effects of Ps-PDRN at the transcriptional level, we analyzed the mRNA expression of pro-inflammatory cytokines in lung tissues by real-time PCR (Figure 3). LPS challenge significantly upregulated TNF-α, IL-1β, and IL-6 mRNA expression compared to the normal control group. Both IN and PO administration of Ps-PDRN markedly suppressed the mRNA levels of these cytokines. Notably, dexamethasone treatment almost completely abolished TNF-α and IL-6 mRNA expression, while Ps-PDRN treatment showed significant but partial inhibition. For IL-1β, all treatment groups demonstrated comparable efficacy in reducing mRNA expression.

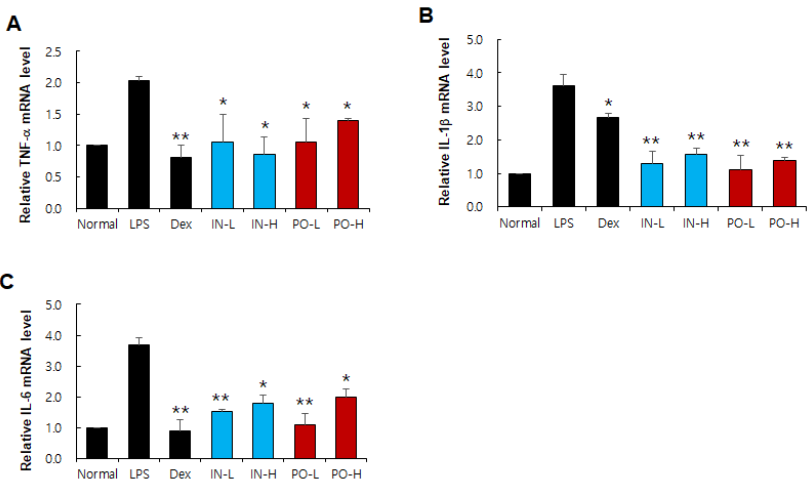


Figure 3. Effects of Ps-PDRN on cytokine mRNA expression in lung tissues. (A) TNF-α; (B) IL-1β; (C) IL-6. mRNA levels were measured by real-time PCR and normalized to GAPDH. Data are presented as mean ± SD (n = 7). * $p < 0.05$, ** $p < 0.01$ vs. LPS group.

3.4. Ps-PDRN Reduces Chemokine Levels in BALF

Chemokines play crucial roles in recruiting inflammatory cells to the lungs during ALI. We measured the levels of MCP-1, RANTES (CCL5), CXCL1, and MIP-2 in BALF (Figure 4). LPS administration dramatically increased the concentrations of all four chemokines compared to the normal group. Treatment with Ps-PDRN significantly reduced the levels of MCP-1, RANTES, CXCL1, and MIP-2 in both IN and PO groups. Notably, oral administration demonstrated superior efficacy

in chemokine suppression, with the PO-L group achieving 100% inhibition of MCP-1 and 97.8% inhibition of MIP-2, surpassing even dexamethasone efficacy (Supplementary Figure S1). The dose-response analysis revealed that oral low-dose treatment showed unexpectedly high efficacy, suggesting an optimal therapeutic window for oral Ps-PDRN administration.

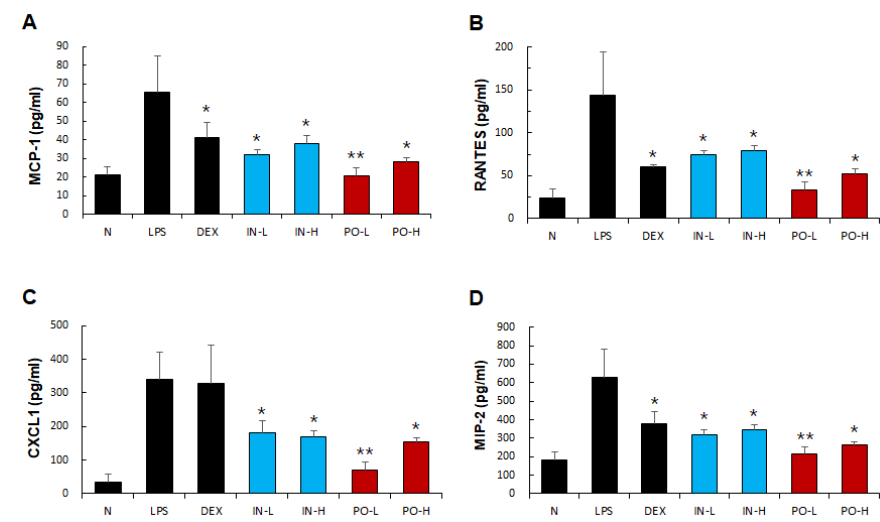


Figure 4. Effects of Ps-PDRN on chemokine levels in BALF. (A) MCP-1; (B) RANTES; (C) CXCL1; (D) MIP-2. Data are presented as mean $\pm$ SD (n = 7). * $p < 0.05$, ** $p < 0.01$ vs. LPS group.

3.5. Ps-PDRN Ameliorates Histopathological Changes in Lung Tissues

Histological examination of lung tissues revealed that LPS administration caused severe pathological changes, including inflammatory cell infiltration, thickening of alveolar septa, and destruction of alveolar architecture (Figure 5). In contrast, treatment with Ps-PDRN via both IN and PO routes markedly attenuated these histopathological alterations. The alveolar structure was better preserved in Ps-PDRN-treated groups, with reduced inflammatory cell infiltration comparable to the dexamethasone-treated group.

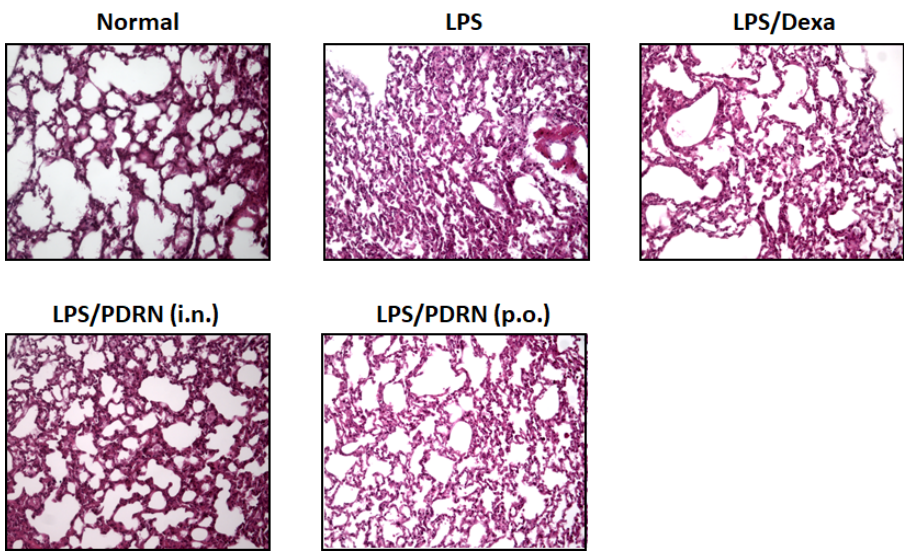


Figure 5. Histopathological analysis of lung tissues (H&E staining). Representative images showing: Normal control with intact alveolar structure; LPS group with severe inflammatory infiltration and alveolar damage; Dexamethasone (DEX) group with reduced inflammation; Ps-PDRN (IN, 50 μ g/mouse) and Ps-PDRN (PO, 200 μ g/mouse) groups showing preserved alveolar architecture and reduced inflammatory cell infiltration.

3.6. Effects of Ps-PDRN on Alveolar Macrophage Activation

Cytological examination of BALF cells revealed distinct morphological changes in alveolar macrophages following LPS challenge (Figure 6). In the LPS group, alveolar macrophages exhibited activated morphology characterized by increased cell size and vacuolization. Treatment with Ps-PDRN reduced the activated morphology of alveolar macrophages, although some degree of activation remained, similar to the dexamethasone-treated group.

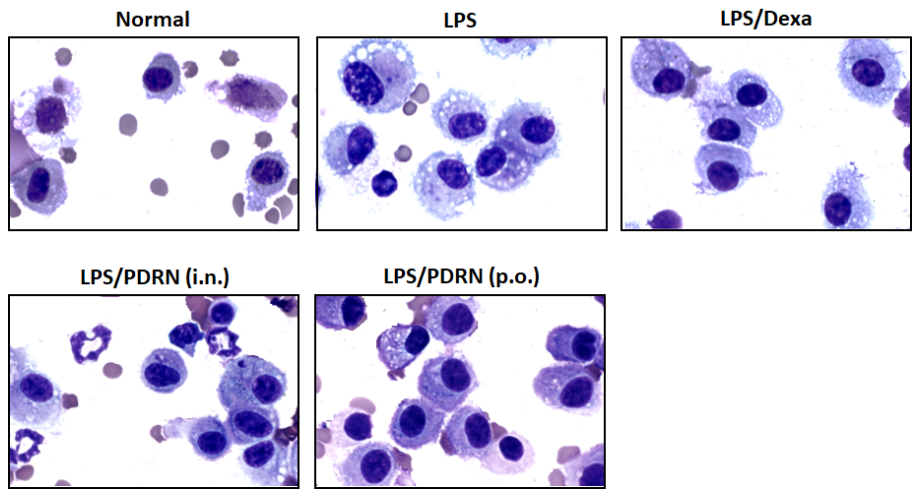


Figure 6. Morphological analysis of alveolar macrophages in BALF. Representative images of Diff-Quik stained BALF cells showing macrophage activation status in Normal, LPS, Dexamethasone (DEX), Ps-PDRN (IN), and Ps-PDRN (PO) groups.

3.7. Ps-PDRN Reduces Serum Inflammatory Markers

To evaluate the systemic anti-inflammatory effects of Ps-PDRN, we measured cytokine and chemokine levels in serum (Figure 7). LPS challenge significantly elevated serum levels of TNF- α , IL-6, MCP-1, and MIP-2. Both IN and PO administration of Ps-PDRN significantly reduced these inflammatory markers. Interestingly, the two administration routes showed distinct efficacy profiles for serum markers: intranasal administration demonstrated superior efficacy in reducing TNF- α (67.0% vs. 51.1% inhibition) and IL-6 (45.5% vs. 30.9% inhibition), while oral administration showed better results for MCP-1 (90.9% vs. 68.2% inhibition) and MIP-2 (86.7% vs. 68.7% inhibition) (Supplementary Figures S2, S4). These results indicate that Ps-PDRN exerts systemic anti-inflammatory effects with route-specific advantages.

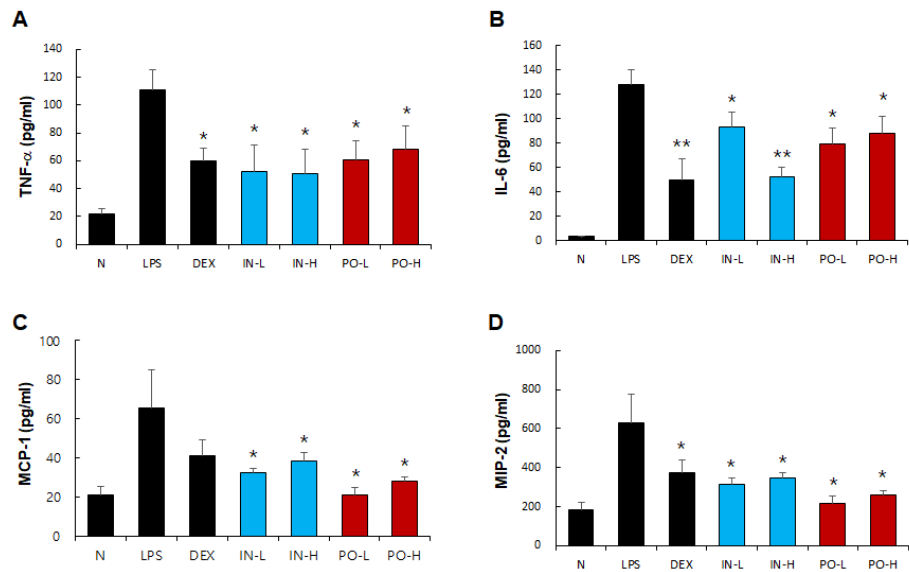


Figure 7. Effects of Ps-PDRN on serum cytokine and chemokine levels. (A) TNF-α; (B) IL-6; (C) MCP-1; (D) MIP-2. Data are presented as mean ± SD (n = 7). * *p* < 0.05, ** *p* < 0.01 vs. LPS group.

3.8. Ps-PDRN Inhibits Inflammatory Responses in RAW 264.7 Macrophages

To investigate the direct anti-inflammatory effects of Ps-PDRN on macrophages, we performed *in vitro* experiments using RAW 264.7 cells. Based on our previous findings that Ps-PDRN inhibits NO production in LPS-stimulated RAW 264.7 macrophages [15], we investigated its inhibitory effects on inflammatory cytokine (TNF-α and IL-6) production here. First, we assessed the cytotoxicity of Ps-PDRN using the MTT assay. Ps-PDRN showed no significant cytotoxicity up to 100 μg/mL in both LPS-stimulated and unstimulated conditions (Figure 8A), although a slight reduction in cell viability was observed at the highest concentration (100 μg/mL) in LPS-stimulated cells. Treatment with Ps-PDRN dose-dependently inhibited LPS-induced production of TNF-α and IL-6 in RAW 264.7 cells (Figure 8B,C). At 25 μg/mL, Ps-PDRN reduced TNF-α production by approximately 40% compared to the LPS control. At 50 μg/mL, TNF-α levels were further reduced to approximately 30% of LPS control levels. Similarly, IL-6 production was significantly inhibited by Ps-PDRN treatment, with the most pronounced effect observed at 50 μg/mL.

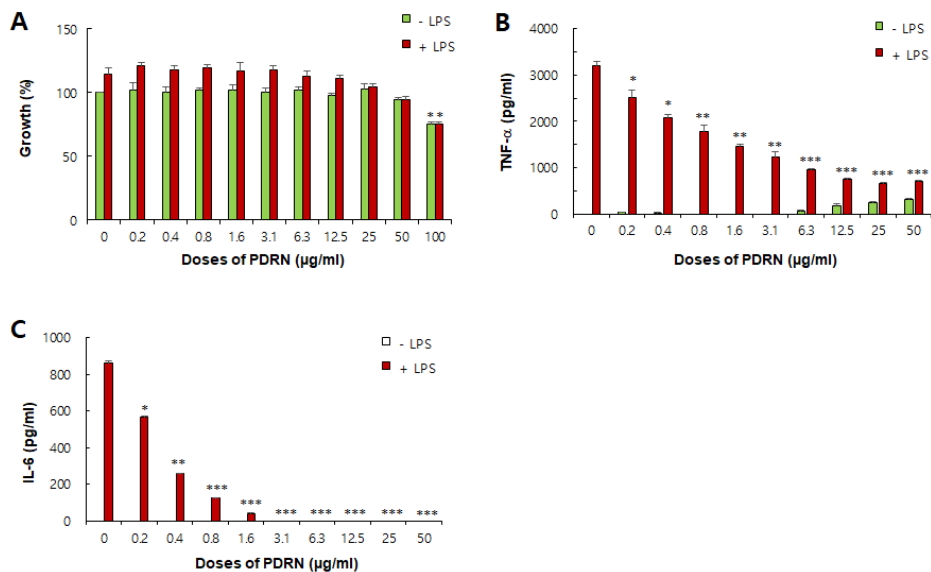


Figure 8. *In vitro* effects of Ps-PDRN on RAW 264.7 macrophages. (A) Cell viability assessed by MTT assay; (B) TNF- α production; (C) IL-6 production. Cells were pretreated with Ps-PDRN for 12 h and stimulated with LPS (1 μ g/mL) for 24 h. Data are presented as mean $\pm$ SD (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. LPS group.

3.9. Ps-PDRN Suppresses Cytokine mRNA Expression in RAW 264.7 Cells

Real-time PCR analysis confirmed that Ps-PDRN suppressed the mRNA expression of pro-inflammatory cytokines in LPS-stimulated RAW 264.7 cells (Figure 9). LPS stimulation dramatically increased TNF- α , IL-1 β , and IL-6 mRNA levels. Treatment with Ps-PDRN (10 and 20 μ g/mL) significantly reduced the mRNA expression of all three cytokines in a dose-dependent manner. At 20 μ g/mL, Ps-PDRN reduced TNF- α , IL-1 β , and IL-6 mRNA levels by approximately 95%, 90%, and 95%, respectively, compared to the LPS control.

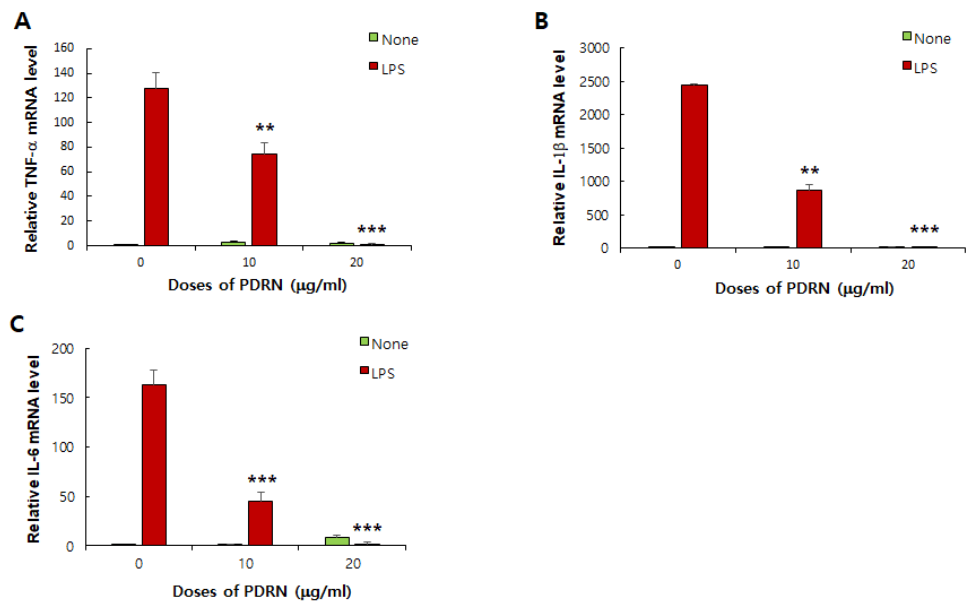


Figure 9. Effects of Ps-PDRN on cytokine mRNA expression in RAW 264.7 cells. (A) TNF- α ; (B) IL-1 β ; (C) IL-6. mRNA levels were measured by real-time PCR and normalized to GAPDH. Data are presented as mean $\pm$ SD (n = 3). ** p < 0.01, *** p < 0.001 vs. LPS group.

4. Discussion

In the present study, we demonstrated for the first time that PDRN derived from *Porphyra* sp. (Ps-PDRN) exerts potent anti-inflammatory effects against LPS-induced ALI in mice. Both intranasal and oral administration routes effectively attenuated systemic and pulmonary inflammation, suggesting versatile therapeutic applications of this marine-derived bioactive compound. ALI is characterized by excessive production of pro-inflammatory cytokines and chemokines that orchestrate the inflammatory cascade leading to lung damage [15,16]. TNF- α , IL-1 β , and IL-6 are key mediators that initiate and amplify the inflammatory response in ALI [17]. Our results showed that Ps-PDRN significantly reduced the levels of these cytokines in both BALF and serum, as well as their mRNA expression in lung tissues. These findings are consistent with previous reports demonstrating the anti-inflammatory properties of salmon-derived PDRN [18,19], suggesting that PDRN from different marine sources shares similar therapeutic mechanisms.

A comprehensive review of PDRN studies from various sources reveals that most investigations have utilized salmon-derived PDRN with molecular weights of 50–1,500 kDa, primarily administered via injection routes (Supplementary Table S2). These studies have demonstrated consistent anti-inflammatory effects through adenosine A₂ A receptor activation across diverse disease models, including wound healing, arthritis, and neurological injuries [8–11,18,19]. In particular, this study is

the first to investigate PDRN derived from *Porphyra* sp., a sustainable marine algae resource other than salmon, and the first to compare intranasal and oral routes of administration of Ps-PDRN in an acute lung injury (ALI) model. The broader molecular weight range of Ps-PDRN (5–1,000 kDa) compared to conventional salmon-derived PDRN may contribute to its distinct pharmacokinetic properties and route-specific efficacy profiles observed in this study.

Chemokines, including not only CC chemokines (MCP-1 and RANTES) but also CXC chemokine (CXCL1 and MIP-2), play crucial roles in recruiting neutrophils and monocytes to the inflamed lungs [20,21]. Excessive neutrophil infiltration is a hallmark of ALI pathogenesis, contributing to tissue damage through the release of reactive oxygen species and proteolytic enzymes [22]. In our study, Ps-PDRN markedly suppressed the production of these chemokines, which correlated with reduced inflammatory cell infiltration observed in histological analysis. This suggests that Ps-PDRN may protect against ALI by limiting the recruitment of inflammatory cells to the lungs.

A notable finding of our study is the distinct efficacy profiles observed between intranasal and oral administration routes (Supplementary Figures S1–S4). Comprehensive comparative analysis revealed that oral administration achieved superior overall inhibition (71.8%) compared to intranasal administration (60.7%), with particularly pronounced differences in chemokine suppression. Oral Ps-PDRN demonstrated remarkable efficacy in inhibiting MCP-1 (92.0% vs. 68.2%), RANTES (81.3% vs. 58.7%), CXCL1 (75.0% vs. 54.8%), and MIP-2 (88.9% vs. 66.7%) in BALF. Conversely, intranasal delivery showed advantages in suppressing certain cytokines, including BALF IL-6 (83.3% vs. 59.7%) and serum TNF- α (67.0% vs. 51.1%) (Supplementary Figure S2). These distinct profiles suggest that the two administration routes may target different aspects of the inflammatory cascade, with oral delivery more effectively suppressing chemokine-mediated immune cell recruitment while intranasal administration preferentially affects local cytokine production. Interestingly, the dose-response analysis revealed unexpected patterns, particularly for oral administration. The PO-L group (100 μ g/mouse) often showed comparable or superior efficacy to the PO-H group (200 μ g/mouse), suggesting a potential therapeutic window beyond which additional dosing may not confer proportional benefits. This phenomenon was most evident for MCP-1 and MIP-2, where PO-L achieved near-complete inhibition (100% and 97.8%, respectively). Such findings have important implications for clinical dose optimization and suggest that lower oral doses may be sufficient for therapeutic efficacy.

Effect size analysis (Cohen's *d*) further quantified the magnitude of Ps-PDRN treatment effects across different markers and administration routes (Supplementary Figure S3). All treatment groups demonstrated large effect sizes ($|d| > 0.8$) for most inflammatory markers, confirming the robust anti-inflammatory activity of Ps-PDRN. The forest plot visualization revealed that oral administration consistently achieved larger effect sizes for chemokines, while intranasal administration showed more pronounced effects on certain cytokines, supporting the concept of route-specific therapeutic profiles.

Intranasal delivery offers the advantage of local drug deposition directly to the respiratory tract, potentially providing faster onset of action and higher local drug concentrations [23]. In contrast, oral administration is more convenient and suitable for chronic management. Our results suggest that Ps-PDRN can effectively reach the lungs and exert anti-inflammatory effects through both routes, offering flexibility in therapeutic applications. The route-specific efficacy profiles may guide clinical decision-making: intranasal administration may be preferred when rapid local cytokine suppression is desired, while oral administration may be advantageous for targeting chemokine-mediated inflammatory cell recruitment.

The *in vitro* experiments using RAW 264.7 macrophages confirmed the direct anti-inflammatory activity of Ps-PDRN to inhibit proinflammatory cytokine production. Macrophages play a key role in the initiation and resolution of inflammation through the secretion of proinflammatory cytokines and chemokines in acute lung injury (ALI) [24]. Our results demonstrated that Ps-PDRN dose-dependently inhibited LPS-induced production of TNF- α and IL-6 proinflammatory cytokines in macrophages without significant cytotoxicity. Furthermore, real-time PCR analysis revealed that Ps-

PDRN suppressed the mRNA expression of pro-inflammatory cytokines, indicating that the anti-inflammatory effects occur at the transcriptional level.

The anti-inflammatory mechanisms of PDRN have been attributed primarily to activation of adenosine A_{2A} receptors, which triggers intracellular signaling pathways that suppress the production of pro-inflammatory mediators [25,26]. Previous studies have shown that salmon-derived PDRN inhibits NF- κ B and MAPK signaling pathways in various cell types [27,28]. Although we did not investigate the molecular mechanisms in the present study, it is reasonable to speculate that Ps-PDRN may exert its anti-inflammatory effects through similar signaling pathways. Future studies are warranted to elucidate the detailed molecular mechanisms underlying the therapeutic effects of Ps-PDRN.

Porphyra sp. represents a sustainable and abundant marine resource for PDRN production. Unlike salmon-derived PDRN, which requires animal-sourced raw materials, *Porphyra* sp. can be sustainably cultivated and harvested without concerns about resource depletion [29]. Additionally, *Porphyra* sp. has a long history of safe consumption as a traditional food in Asian countries, suggesting a favorable safety profile for therapeutic applications [30]. These advantages make *Porphyra* sp.-derived PDRN an attractive alternative to conventional PDRN sources.

There are several limitations of this study that should be acknowledged. First, we did not investigate the specific molecular mechanisms by which Ps-PDRN exerts its anti-inflammatory effects, including A_{2A} receptor activation and downstream signaling pathways. Second, the study focused on a prevention model where Ps-PDRN was administered before LPS challenge; therapeutic efficacy in an established ALI model remains to be determined. Third, long-term safety and pharmacokinetic profiles of Ps-PDRN require further investigation. Despite these limitations, our findings, supported by comprehensive comparative analysis of administration routes, provide strong evidence for the therapeutic potential of Ps-PDRN in inflammatory lung diseases.

5. Conclusions

In conclusion, we demonstrated that PDRN derived from *Porphyra* sp. effectively ameliorates LPS-induced acute lung injury in mice through both intranasal and oral administration routes. Ps-PDRN significantly attenuated fever, pulmonary edema, and inflammatory cell infiltration while reducing the production of pro-inflammatory cytokines and chemokines. Notably, our comparative analysis revealed distinct efficacy profiles between the two administration routes, with oral administration showing superior chemokine suppression and intranasal delivery demonstrating advantages in certain cytokine inhibition. The *in vitro* studies confirmed the direct anti-inflammatory activity of Ps-PDRN on macrophages. These findings suggest that *Porphyra* sp.-derived PDRN is a promising natural therapeutic agent for the prevention and treatment of inflammatory lung diseases. Further studies are needed to elucidate the molecular mechanisms and to evaluate the clinical potential of this marine-derived bioactive compound.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplementary Figure S1: Inhibition rate heatmap of inflammatory markers; Supplementary Figure S2: Radar chart comparison of intranasal vs. oral administration efficacy profiles; Supplementary Figure S3: Effect size (Cohen's d) forest plot analysis; Supplementary Figure S4: Comparative analysis of administration routes by sample type; Supplementary Table S1: Summary of PDRN Studies from Different Marine Sources.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

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