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Protectin DX promotes the inflammatory resolution via activating COX-2/L-PGDS-PGD₂ and DP₁ receptor in acute respiratory distress syndrome

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A B S T R A C T

Purpose: Acute respiratory distress syndrome (ARDS) is characterized by uncontrollable inflammation. Cyclooxygenase-2 (COX-2) and its metabolite prostaglandins are known to promote the inflammatory resolution of ARDS. Recently, a newly discovered endogenous lipid mediator, Protectin DX (PDX), was also shown to mediate the resolution of inflammation. However, the regulatory of PDX on the pro-resolving COX-2 in ARDS remains unknown.

Material and Methods: PDX (5 μg/kg) was injected into rats intravenously 12 h after the lipopolysaccharide (LPS, 3 mg/kg) challenge. Primary rat lung fibroblasts were incubated with LPS (1 μg/ml) and/or PDX (100 nM). Lung pathological changes examined using H&E staining. Protein levels of COX-2, PGDS and PGES were evaluated using western blot. Inflammatory cytokines were tested by qPCR, and the concentration of prostaglandins measured by using ELISA.

Results: Our study revealed that, COX-2 and L-PGDS has biphasic activation characteristics that LPS could induce COX-2 and L-PGDS during resolution of Acute Lung Injury. The secondary peak of COX-2, L-PGDS-PGD₂ promoted the inflammatory resolution in ARDS model with the DP₁ receptor being activated and PDX up-regulated the inflammatory resolution via enhancing the secondary peak of COX-2/L-PGDS-PGD₂ and activating the DP₁ receptor.

Conclusion: PDX promoted the resolution of inflammation of ARDS model via enhancing the expression of secondary peak of COX-2/L-PGDS-PGD₂ and activating the DP₁ receptor. PDX shows promising therapeutic potential in the clinical management of ARDS.

A R T I C L E  I N F O

Keywords: Acute respiratory distress syndrome, Cyclooxygenase-2, Lipocalin-type prostaglandin D synthase, Protectin DX, Inflammatory resolution

1. Introduction

Acute respiratory distress syndrome (ARDS) is a severe acute inflammatory disease, caused by various factors like pneumonia, aspiration of gastric contents and sepsis. [1–2] Main pathological damages of ARDS were alveolar epithelial and lung endothelial barrier injury, resulting in the accumulation of protein-rich edema fluid in the alveolar space. [3] ARDS lacks of effective pharmacological treatment. [4] The resolution of inflammation serves as the self-protected character in host and is an initiative process. Therefore, the active inflammatory resolution may become a clue to find out effective therapeutic method for ARDS.

COX-2, and prostaglandins, are associated with a variety of inflammatory diseases. In ARDS patients’ bronchoalveolar fluid, PGs levels were found to be increased. [5], However, inhibition of COX-2 has not been proven being clinically effective in ARDS [6]. In animal studies, pharmacologic inhibition or gene silencing of COX-2 would block the inflammatory resolution of Acute Lung Injury. [7] In the mouse model of carrageenin-induced pleurisy, COX-2 promotes resolution by generating pro-resolving prostaglandins. [8] Moreover, COX-2 is responsible for the producing of pro-resolving PGs, like PGD₂, 15-deoxy-D12,14-PGJ₂, and PGF₃α. [9–10] These works suggest that COX-2 may play both pro-

Abbreviations: ARDS, Acute respiratory distress syndrome; COX-2, Cyclooxygenases-2; H-PGDS, Hematopoietic prostaglandin D synthase; L-PGDS, Lipocalin-type prostaglandin D synthase; LPS, Lipopolysaccharide; mPGES-1, Microsomal prostaglandin E synthase-1; mPGES-2, Microsomal prostaglandin E synthase-2; PDX, Protectin DX; PGs, Prostaglandins; PGE₉, Prostaglandin E2; PGD₂, Prostaglandin D2; SPM, Specialized pro-resolving lipid mediators.

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inflammatory and pro-resolving roles in inflammatory diseases like ARDS.

Prostaglandins were induced by COXs, especially COX-2 when in the presence of inflammation. Prostaglandin E2 (PGE2) was normally considered to be a pro-inflammatory PG. Previous study showed that administration of PGE2 or its receptor agonist improved lung function in mice ALI model. Another prostanoid, prostaglandin D2 (PGD2) ameliorated lung injury in ALI/ARDS model via enhancing the endothelial barrier repairment. Both PGE2 and PGD2 are converted from PGH2 by various synthases. PGE2 synthases consist of cPGE, microsomal prostaglandin E synthase-1 (mPGE1) and microsomal prostaglandin E synthase-2 (mPGE2). Various pro-inflammatory stimulations can up-regulate mPGE1, m. Meanwhile, cPGE and mPGE2 are constitutively expressed. PDX acts its important role via G protein-coupled receptor DP1 (DP1) and the chemoattractant receptor-homologous molecule CRTH2 (DP2). PDX showing multiple pathophysiological characters via different receptors.

Primary lung fibroblasts, which are far from being bystander cells, are important to host defense in ARDS. After the inflammatory stimulation, fibroblasts are activated following the immune response, and secreting a large number of cytokines like interleukin 6 (IL-6) and interleukin 8 (IL-8). Growing evidence manifested that fibroblasts-secreted growth factors promote the alveolar barrier functions and alleviate the lung injury induced by LPS. Our pervious study suggested that fibroblasts regulate the inflammatory resolution by producing proresolving mediators PGD2.

Formal studies have reported that endogenous lipid mediators and mechanisms can drive the resolution of inflammation the resolution of inflammation was driven by novel lipid mediators and endogenously triggered mechanisms. Specialized pro-resolving lipid mediators (SPM) were identified as new genus, including Resolvins, Protectins and their aspirin-triggered forms. Protectin DX (10S,17S-dihydrodocos-4Z,7Z,11E,13Z,15E,19Z hexaenoic acid) is a newly discovered member of this genus, which derived from natural ω-3 fatty acid docosahexanoic acid (DHA). PDX possesses anti-inflammatory and inflammation pro-resolving bioactivities. A study reported that PDX maintains the integrity of lung epithelium, increases the alveolar fluid clearance of ARDS in rat. PDX regulates inflammatory cell infiltration via resident macrophage in LPS-induced lung injury. Moreover, PDX was shown to alleviate lung injury induced by LPS via inducing primary rat type II alveolar epithelial cells proliferation and inhibiting their apoptosis in vivo and in vitro.

Our studies confirmed that COX-2 has a biphasic activation pattern in LPS stimulated lung fibroblasts, showing that COX-2 and PGD2 expression levels peaked at 6 h and subsequently after 48 h. Moreover, NF-kB p50/50 was responsible for regulating the secondary expression peak of COX-2 in the resolution stage. However, the downstream mechanism of secondary peak COX-2 and PGD2 in the resolution of inflammation remains unclear. Further, whether PDX promotes the resolution of inflammation by regulating secondary peak of the COX-2 and PGD2 has not been proved yet.

In this study, we hypothesize that the secondary peak of COX-2/L-PGDS-PGD2 promote the resolution of inflammation in the ARDS model. Moreover, we surmise that PDX plays a pro-resolving role in ARDS by enhancing the pro-resolving COX-2/L-PGDS-PGD2 expressions and activating the DP1 receptor.

2. Materials and Methods

2.1. Reagents

Protectin DX, NS-398(selective COX-2 inhibitor), AT-56 (L-PGDS inhibitor), BW245C (DP1 receptor agonist), 15(R)-15-methyl-PGD2(CRTH2/DP2 receptor antagonist), NS-398 (selective COX-2 inhibitor), AT-56 (L-PGDS inhibitor), BW245C (DP1 receptor agonist), 15(R)-15-methyl-PGD2(CRTH2/DP2 receptor antagonist), and CAY-10471(CRTH2/DP2 receptor antagonist) were obtained from Cayman Chemical (Ann Arbor, MI, USA), Lipopolysaccharide (Escherichia coli O55: B5), BOC-2 (ALX/FPR2 receptor inhibitor) were purchased from Biomol/Enzo Life Sciences (Farmingdale, NY, USA).

2.2. Animal procedures

Male Sprague Dawley (SD) rats (200–250 g) were purchased from SLAC Laboratory (Shanghai, China). SD rats were raised in a temperature-controlled room (22–24 °C) on a 12 h day/night cycle with free access to food and water. All animal experimental procedures were approved by the Animal Care and Use Committee Institutional of Wenzhou Medical University (Wenzhou, China).

SD rats were injected with 3 mg/kg body weight of LPS intravenously, meanwhile, control group animals were administered the same volume of sterile 0.9% saline. Rats were euthanized at the different time points: 0, 6, 12, 24, 48 and 72 h after LPS stimulation.

For experimental procedures, NS-398, BOC-2, AT-56, BW-245C, 15(R)-15-methyl-PGD2, MK-0524 and CAY-10471 were dissolved in DMSO and then diluted into sterile 0.9% saline for further use. At 12 h after the LPS challenge, rats were given PDX (5 μg/kg) or an equivalent volume of ethanol via tail vein injection. Rats were administered NS-398 (5 mg/kg) i.v. 1 h prior than the LPS challenge or 12 h after the LPS administration. AT-56 was given intravenously (5 mg/kg) 12 h after the LPS challenge. For DP receptors studies, BW-245C, 15(R)-15-methyl-PGD2, MK-0524 and CAY-10471 were injected (5 μg/kg) i.p. to rats 12 h after the LPS exposure. BOC-2 (600 ng/kg) were given i.v. to rats 1 h prior to PDX injection, whereas other groups received an equal volume of DMSO/saline solution. Rats were sacrificed at 24 h humanely under anesthesia.

2.3. Western blot analysis

Rat primary lung fibroblasts and lung tissues were washed in iced PBS and harvested by using RIPA buffer supplemented with protease inhibitors. Resulting supernatant fraction was homogenized in 1 × SDS–PAGE sample buffer and boiled for 10 min at 95 °C. For the immunoblotting, protein lysates were electrophoresed via 10%,12% or 15% SDS–PAGE gels and then transferred to polyvinylidene fluoride (PVDF) membranes. Membranes were blocked and incubated with the indicated primary antibody overnight at 4 °C. Primary antibodies against COX-2 (ab-52237), L-PGDS (ab-182141) were purchased from Abcam (Cambridge, UK), mPGE1 antibody (DF-8592) and mPGE2 antibody (DF-12712) were purchased from Affinity (Cincinnati, OH, USA). Bound primary antibodies were incubated with appropriate secondary antibodies for another 1 h. Protein levels were detected by using chemiluminescence reagents from Thermo Scientific (Rockford, IL, USA). Images were scanned by a UVP imaging system and analyzed by the Image Quant LAS 4000 mini system (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).

2.4. Cell culture

Primary lung fibroblasts were isolated from SD rats; isolation process was same as we described before. For experimental procedures, fibroblasts (5 × 105) were plated in six wells plates and grown to 80% confluence. Pulmonary fibroblasts were allowed to remain in a quiescent state for 24 h by incubating them in medium containing 1% FBS (Life Technologies BRL; Grand Island, NY, USA) prior to experimental treatment. After 24 h of culture with LPS (1 μg/ml) or a control medium, fibroblasts were treated with 1,500 nM PDX or a vehicle solution (0.1% ethanol, as the PDX was supplied in ethanol) for an additional 24 h. BOC-2 (10 μM) was added 30 min before PDX administration. Fibroblasts were harvested at the different time points: 0, 6, 12, 24, 48 and 72 h after LPS challenge for further use.
2.5. Histopathological staining

The same part of the left lung of each rat was fixed in 10% paraformaldehyde for 24 h. Lung tissues were embedded in paraffin wax, sectioned, and stained with H&E for light microscopy analysis. Acute lung injury scores were quantified by a single observer who was blinded to the treatment groups via the established histopathological scoring system. [31]

2.6. Elisa

PGE$_2$ and PGD$_2$ concentrations in fibroblasts cellular supernatants and rat homogenized lung tissues were measured as previously described. [32] ELISA kits were purchased from R&D Systems (Minneapolis, MN, USA), all procedures were performed according to the manufacturers’ instructions. All analyzes were run in triplicate and repeated twice.

2.7. Quantitative real-time PCR

Total RNA samples in lungs were isolated using TRIzol reagent (Takara Bio, Kusatsu, Japan) according to the manufacturer’s protocol. The cDNA of mRNA was synthesized by the reverse transcription kit purchased from Thermo Scientific (Rockford, IL, USA). The expression of mRNA was detected by qPCR (Bio-Rad, Hercules, CA, USA) with TB Green® Premix Ex Taq™ PCR kit (Takara Bio, Kusatsu, Japan). The gene-specific primers used are listed in Table S1 and mRNA levels normalized to GAPDH. Data were calculated with using the 2-
2.8. Statistical analysis

All data were presented as mean ± SEM. All data were analyzed using one-way ANOVA, followed by a Tukey test for post hoc comparisons. P < 0.05 was considered as significant. Statistical analyses were performed using GraphPad Prism 8.0 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Biphasic activation of COX-2, L-PGDS in rat primary lung fibroblasts stimulated by LPS

Pulmonary lung fibroblasts play an important role in inflammatory diseases and participate actively in immune response. [33] Herein, to find out the dynamic change of COX-2 expression in fibroblasts, cells were exposed to LPS for 0, 6, 12, 24, 48, 72 h (Fig. 1A). We found that the COX-2 protein presents a biphasic expression character, COX-2 firstly peaked at 6 h and secondary one at 48 h (Fig. 1B).

Interestingly, we found that the L-PGDS also showed a biphasic expression, similar to that of COX-2 expression, peaking at 6 and 48 h (Fig. 1C). Moreover, mPGES-1 only presented a single peak expression at 6 h (Fig. 1D). mPGES-2, as a constitutively expressed synthetase, [34]...
showed no significant changes after the LPS challenge (Fig. 1E). PGE$_2$, as a pro-inflammatory prostaglandin, [35] highly expressed at 6 h only (Fig. 1F). In contrast, PGD$_2$ highly peaked both at 6 h and 48 h, and the secondary peak of PGD$_2$ was significantly higher than the first one (Fig. 1G). We revealed that there was a biphasic activation character of COX-2/L-PGDS-PGD$_2$ in LPS-stimulated lung fibroblasts.

3.2. PDX enhances the secondary peak of COX-2, L-PGDS via activating the ALX receptor in vitro

To determine the effect of Protectin DX on the secondary peak of COX-2/L-PGDS expression. COX-2 and L-PGDS protein levels were tested at 48 h with the treatment of PDX (Fig. 2A). Our results demonstrated that PDX enhanced COX-2 and L-PGDS protein levels in a dosage-dependent pattern (Fig. 2B and 2C).

We have previously demonstrated that PDX ameliorates the wound repair of the lung epithelial barrier via ALX receptor. [27] Here we would like to know whether PDX enhances the secondary peak of COX-2/L-PGDS via ALX receptor. The ALX receptor antagonist, BOC-2(10 μM) was added 30 min before the PDX treatment. As Fig. 2D and Fig. 2E presented, pre-treatment with BOC-2 reversed the promoting effect of PDX on the secondary peak of COX-2 and L-PGDS, suggesting the promoting effect of PDX on the expression of secondary peak COX-2, L-PGDS are via the ALX receptor.
3.3. Biphasic activation of COX-2 and L-PGDS in LPS-stimulated ARDS murine model

Next, to explore the activation character of COX-2 and L-PGDS in vivo, we established the self-limited ARDS model in SD rats by administrating low dosage of LPS intravenously (Fig. 3A). Pathomorphological changes were detected by H&E staining, compared with the control group, the lung architecture in the LPS group showed most remarkable damage at 12 h, as evidenced by the changes in lung injury score. The mRNA expression of IL-1β, IL-6, and TNF-α (G and H) Relative expression level of L-PGDS and mPGES-1 protein in the lung was determined by western blot. (I and J) The concentrations of PGE₂ and PGD₂ in lungs were detected by ELISA. Data are presented as mean ± SEM, n = 6. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
Our results also showed L-PGDS had a biphasic expression characteristic in vivo as same as in fibroblasts. The expression of L-PGDS firstly increased at 6 h, and the secondary peak appeared at 24 h (Fig. 3C), mPGES-1 was highly expressed at 6 h only (Fig. 3D). mPGES-2 showed no significant change after the LPS stimulation (Fig. 3E). The production of PGE$_2$ increased only at 6 h and then gradually decreased, in consistent with the mPGES-1 expression (Fig. 3F). PGD$_2$ levels peaked at both 6 and 24 h, in consistent with the L-PGDS expression (Fig. 3G).

Fig. 5. L-PGDS secondary peak promotes the inflammatory resolution in vivo. (A) SD rats were administered with LPS (3 mg/kg) or the same volume of sterile intravenously, AT-56 (5 mg/kg), or the same volume of 0.9% saline was injected i.v. to rats 12 h after LPS injection. (B) Pathomorphological staining of the lung tissues. (Original magnification, 200X; inset, 400X). (C) Acute lung injury scores. (D–F) The mRNA expression levels of inflammatory cytokines: IL-1β, IL-6, TNF-α were measured by qPCR. (G and H) PGE$_2$ and PGD$_2$ concentrations in lung tissues were measured by ELISA. Data are shown as mean ± SEM, n = 6. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
Fig. 6. PDX promotes the inflammatory resolution by enhancing the activation of ALX/FPR2 receptor and the COX-2-L-PGDS-PGD2 expression in vivo. (A). SD rats were administered with LPS (3 mg/kg) or the same volume of sterile intravenously, PDX (5 μg/kg) were administered intravenously to rats at 12 h after LPS treatment, BOC-2 600 ng/kg was given for 1 h before PDX treatment. Lung tissues were collected at 24 h. (B) Representative pathological H&E staining sections of lung tissues. (Original magnification, 200x; inset, 400x). (C) Acute lung injury scores assessment of each group. (D–F) The mRNA expression level of inflammatory cytokines: IL-1β, IL-6, TNF-α. (G and H) Expression levels of COX-2 and L-PGDS protein after PDX treatment was determined by western blot analysis. (I and J) Concentration of PGE2 and PGD2 were detected by ELISA. All data are presented as mean ± SEM, n = 6. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
These findings uncovered that there has a biphasic expression feature of COX-2/L-PGDS-PGD2 in ARDS rat model. COX-2 might be proinflammatory at early stage (via the mPGES-1/PGE2 expression). Secondary peak of COX-2 might be pro-resolving in the resolution stage (via L-PGDS/PGD2).

3.4. The effect of COX-2 secondary peak on inflammation resolution of the rat ARDS model

Next, to determine if secondary peak of COX-2 could play a pro-resolving role in the resolution of ARDS model. NS-398, a clinical-used selective COX-2 inhibitor, was administrated intravenously in rats 1 h before (the NS-398 + LPS group) or at 12 h (the LPS + NS-398 group) after the LPS challenge (Fig. 4A). Pathological features were detected at 24 h as shown in Fig. 4B. Compared with the NS-398 + LPS group and LPS group, the LPS + NS-398 group revealed more distinct interstitial edema, hemorrhaging, thickening of alveolar walls, and inflammatory cells infiltration in the lung tissues. In contrast to the LPS group, the NS-398 + LPS group showed less pathological damage. As shown in Fig. 4C, acute lung injury score was quantified and found to be consistent with the pathophysiological changes. In addition, compared with the NS-398 + LPS group and the LPS group, relative mRNA levels of the proinflammatory cytokines: IL-1β, IL-6 and TNF-α were much higher in LPS + NS-398 group (Fig. 4D-F). These findings suggested that inhibition the latter peak of COX-2 postponed the resolution of inflammation.

Moreover, the COX-2 inhibition significantly decreased the protein expression of L-PGDS at 24 h. No significant difference of L-PGDS protein expression was observed between the LPS + NS-398 group and the LPS group (Fig. 4G). Pre-treatment of NS-398 decreased the protein level of mPGES-1 in ARDS model (Fig. 4H), no significant change in the PGE2 production was observed between LPS group and the NS-398 + LPS group (Fig. 4I). Inhibition of the COX-2 reduced the production of PGD2 at 24 h (Fig. 4J).

In summary, inhibition of the secondary peak COX-2 in the resolution stage of inflammation caused more significant lung damages compared to the inhibition of the first peak of COX-2 in the early stage of inflammation. Based on these findings, we surmise that the secondary peak of COX-2 plays a key role in promoting the resolution of inflammation in ARDS.
3.5. L-PGDS secondary peak promotes the inflammatory resolution in vivo

We then attempted to figure out the function of the secondary peak of L-PGDS. AT-56 was administrated to rats at 12 h after the LPS challenge (Fig. 5A). H&E staining (Fig. 5B) revealed that, compared with the LPS group, LPS + AT-56 group displayed more interstitial edema, hemorrhage, and inflammatory cells infiltration in lung tissues. In accordance with this, the lung injury scores were also elevated (Fig. 5C), along with the release of IL-1β, IL-6, and TNF-α (Fig. 5D–F). These results indicated that suppression of L-PGDS blocked ARDS resolution. ELISA results also proved that inhibiting the L-PGDS decreased the PGD₂ production at 24 h (Fig. 5H), while no significant difference was found in PGE₂ between the LPS group and the LPS + AT-56 group (Fig. 5G).

All results above indicated that the secondary peak COX-2/L-PGDS-PGD₂ was responsible for ARDS resolution in murine models.

3.6. PDX promotes inflammatory resolution by enhancing the activation of ALX receptor and the COX-2/L-PGDS-PGD₂ expressions in vivo

We have already been proved to enhance the repair of lung epithelial barrier in ALI/ARDS murine model. To evaluate whether PDX promotes inflammatory resolution via activating ALX receptor and the COX-2/L-PGDS-PGD₂ expressions in rat ARDS model, PDX was given at 12 h after the LPS exposure (Fig. 5A). H&E staining result showed that PDX markedly alleviated the morphological and histological damages induced by LPS, consistent with a decrease in acute lung injury score (Fig. 5B and 5C). Administration of BOC-2 (the ALX receptor inhibitor) reversed the effect of PDX on both histological damages and the release of pro-inflammatory cytokines induced by LPS (Fig. 5D–F), consistently with the acute lung injury score (Fig. 5C). These findings suggested that PDX promotes the inflammation resolution via activating the ALX receptor.

In addition, PDX significantly up-regulated the protein expression of COX-2 and L-PGDS during the resolution stage. PDX significantly promoted the expression of the pro-resolving mediator PGD₂ (Fig. 5J). No significant difference was found in the PGE₂ level between the LPS group and the LPS + AT-56 group.
and the LPS + PDX group (Fig. 6I). Pre-stimulation with BOC-2 reversed the improved effect of PDX on COX-2 and L-PGDS protein expression as well as PGD2 secretion (Fig. 6G -H). These results indicated that PDX facilitated the inflammatory resolution via activating ALX receptor and enhancing the expression of COX-2/L-PGDS as well as the production of PGD2.

3.7. DP1 receptor is activated during the resolution phase in the murine ARDS model

Previous studies demonstrated that the PGD2-DP1 signaling pathway was responsible for the anti-inflammatory function of ALI/ARDS. [12-13] Herein, we investigated what kind of PGD2 receptor plays the major role during the resolution phase in the murine ARDS model, BW-245C (DP1 receptor agonist), 15(R)-15-methyl-PGD2(CRTH2/DP2 receptor agonist), MK-0524(DP1 receptor antagonist) or CAY-10471 (CRTH2/DP2 receptor antagonist) was intraperitoneally injected into rats at 12 h after the LPS stimulation (Fig. 7A). Morphological staining (Fig. 7B) revealed that the DP1 receptor agonist BW-245C significantly alleviated the pathological damage, while the DP1 receptor antagonist (MK-0524) aggravated the lung injury. In comparison, the agonist or antagonist of the DP2 receptor did not significantly influence the lung injury condition. As expected, the acute lung injury scores were in line with the findings of morphological staining (Fig. 7C). Furthermore, mRNA levels of pro-inflammatory cytokines: IL-1β (Fig. 7D), IL-6 (Fig. 7E), TNF-α (Fig. 7F) also validated the above results.

3.8. PDX promotes the inflammatory resolution through the activation of the DP1 receptor in the rat ARDS model

Then, we investigated if PDX promoted the inflammatory resolution through the activation of the PGD2 receptors in the rat ARDS model. BW-245C, 15(R)-15-methyl-PGD2, MK-0524 or CAY-10471 were given to rats with or without PDX (Fig. 8A). Pathological staining (Fig. 8B) showed that the DP1 receptor agonist enhanced the pro-resolving function of PDX, while the DP1 receptor antagonist suppressed the improved inflammatory resolution by the PDX treatment. Meanwhile, the DP2 receptor did not significantly affect the resolution of inflammation promoted by PDX (Fig. 8B). The acute lung injury scores were in accordance with the findings of morphological staining (Fig. 8C). Furthermore, inflammatory cytokines levels (IL-1β, IL-6 and TNF-α) were down-regulated after the DP1 receptor agonist treatment, while up-regulated by DP1 receptor antagonist (Fig. 8D-F). In contrast, the DP2 receptor did not affect the release of IL-1β, IL-6 and TNF-α (Fig. 8D-F). Altogether, these data suggested that PDX promotes resolution of inflammation by activating the DP1 receptor in vivo.

4. Discussion

Our study uncovered that COX-2/L-PGDS-PGD2 expressions have a dual activation induced by LPS. Importantly we found that the secondary peak of COX-2/L-PGDS-induced PGD2 was responsible for the pro-resolving process in ARDS. Moreover, we showed that the DP1 receptor was activated in inflammatory resolution. This study provides evidence for a new mechanism by which PDX may promote inflammation resolution of the ARDS model through improving the expression of the secondary peak of COX-2/L-PGDS-induced PGD2. Interestingly, the ALX receptor antagonist, BOC-2, abrogated the effect of PDX on the COX-2/L-PGDS-PGD2. Altogether, these findings were summarized in Fig. 9, showed that PDX also promotes the inflammatory resolution via activating the ALX receptor and enhances the inflammatory resolution partly via activating the DP1 receptor.

COX-2 is catalyzed after the inflammatory stimuli immediately. Pro-inflammatory PGs were induced by COX-2. [36] Fukunaga K. et al indicated that COX-2 plays a protective role in ALI/ARDS through COX-2-derived mediators, partly via enhancing the lipoxin signaling. [7] However, this study did not discuss the dynamic change of COX-2 expression in the ARDS model. In our LPS-stimulated ARDS model, we revealed that COX-2 was quickly and peaked at 6 h, then peaked twice at 24 h in vivo. The secondary peak of COX-2 displayed pro-resolving character which was distinguished from traditional concepts. Consistent with our study, Gilroy D.W. et al reported the biphasic expression of COX-2 in the carrageenin-induced pleurisy mouse model. [8] However, the specific role of each COX-2 peak remains unclear. Herein, our findings suggested that COX-2 could be proinflammatory at early stage and be pro-resolving during the later stage of inflammation. Therefore, blindly using COXs inhibitors such as NSAIDs may postpone the resolution of ARDS. Administration of the agonist or antagonist of the DP1 receptor was activated in the resolution of ARDS. Administration of the agonist of the DP1 receptor improved the resolution of inflammation,
while the inhibition of the DP₁ receptor aggravated lung injuries. In our self-limited ARDS model, activation or inhibition of DP₂ receptors did not affect the resolution of inflammation.

As previously described, the resolution of acute inflammatory diseases is an active process. [38] There is an internal, self-protective feature among the patient with ARDS. Thus, we established a self-limited ARDS model for further study. PDX, as an endogenous “braking signal”, displays anti-inflammatory and pro-resolving characteristics. We already have determined that PDX could ameliorate the braking signal limited ARDS model for further study. PDX, as an endogenous PGDS-and its metabolite PGD₂, contributes to the resolution of acute lung injury, J. Pathol. 248 (3) (2009) 285-300. Therefore, we found that the activation of the COX-2/PGDS secondary peak and the DP₁ receptor may be the novel mechanism by which PDX exerts its pro-resolving effect on inflammation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Disclosure

The authors declare that they have no conflicts of interest for this work.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2021.108348.

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