Original Article Activation of cannabinoid receptor type 2 reduces lung ischemia reperfusion injury through PI3K/Akt pathway

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Abstract: Cannabinoid receptor-2 activation plays a protective role against ischemic reperfusion injury (IRI) in various organs, and exerts a protective effect against paraquat-induced acute lung injury, while the role of CB, in lung IRI remains unclear. Hence, the present study was designed to explore the role of CB, in lung IRI, and whether the PI3K pathway was involved. C57BL/6 mice were subjected to lung ischemia by clamping the left hilum for 1 hour, followed by 2 hours' reperfusion. Mice were pretreated with vehicle, CB, agonist JWH133, or antagonist AM630 followed by JWH133. Arterial blood and left lung tissues were collected to detect the PaO,/FiO, ratio, lung wet-to-dry weight ratio, lung pathologic scoring, pro-inflammatory cytokines, MDA, and SOD. Secondly, mice were pretreated with vehicle, JWH133, or both PI3K-inhibitor LY294002 and JWH133. Arterial blood and left lung tissues were collected for the above studies and protein expression of CB₂ receptor, p-AKT, and AKT. After mice were pretreated with JWH133, IR-induced lung edema and lung histopathologic changes were significantly attenuated. Pretreatment with JWH133 improved PaO₂/FiO₂ ratio, decreased lung TNF-α, IL-6, MDA levels and MPO activities, and increased SOD activity. By contrast, the protective effect of JWH133 was blocked by pretreatment with CB₂ antagonist AM630. Similarly, pretreatment with PI3K-inhibitor weakened the protection induced by JWH133, and downregulated the expression of p-AKT without altering CB, expression. The study suggested that activation of CB, receptor plays a protective role against IR-induced lung injury through reducing inflammation in mice. The PI3K/Akt pathway might be involved in the protective effect of CB₂ receptors in lung IRI.

Keywords: Cannabinoid receptor-2, lung injury, ischemic reperfusion injury, PI3K/Akt pathway

Introduction

It is well-known that lung ischemic reperfusion injury (IRI) remains a common and severe postoperative complication following cardiopulmonary bypass, lung transplantation, pulmonary embolism, and cardiac arrest [1, 2], with high morbidity and mortality. Inflammatory response, oxidative stress, intracellular calcium overload, and neurogenic inflammatory pathways are important contributors to lung IRI [3, 4]. Unfortunately, these underlying mechanisms have not yet been completely elucidated.

It has been showed that cannabinoid (CB) receptors distributed in rat and human pulmonary artery endothelial cells can be activated to alleviate oxidative stress and the inflammatory response [5]. Cannabinoid receptors, composing the endocannabinoid system (ECS) with endogenous cannabinoids, and enzymes responsible for the synthesis and degradation of endocannabinoids [6], mainly include cannabinoid receptor-1 (CB₁ receptor), mainly expressed in the central nervous system, and cannabinoid receptor-2 (CB₂ receptor), expressed in the immune system, lung, spleen, reproductive system [7].

Previous studies demonstrated that activation of CB_2 receptors had a protective role against tissue damage associated with inflammation and oxidative stress in various organs, such as lung, heart, liver, brain, lung, and bladder [8-10]. CB_2 agonist JWH015 decreased leukocyte infiltration and myeloperoxydase activity in LPSinduced interstitial cystitis [9]. Liu et al. indicated that CB_2 agonist JWH133 generated a protective effect against paraquat-induced acute lung injury by inhibiting inflammation [10]. Moreover, our research group has reported that enzymes synthesizing and degrading endocannabinoids were involved in lung IRI [11]. However, the effect of CB_2 on lung ischemic-reperfusion injury remains unknown.

Phosphatidylinositol 3 kinase (PI3K/Akt) pathway plays a vital role in the process of cell survival, differentiation, and proliferation. Li et al. found that activation of CB2 receptors prevented apoptosis induced by myocardial IRI through PI3K/Akt pathway [12]. PI3K/Akt pathway also involved in acute lung injury (ALI). In LPS-induced ALI model, Allicin ameliorated inflammation, oxidative stress, and apoptosis by the PI3K/Akt pathway in neonatal rats [13]. Administration of high dose dexmedetomidine before ischemia could protect against lung IRI, and the PI3K/Akt pathway was involved [14]. Thus, this study was designed to investigate the potential protective role of CB₂ receptors in a murine model of lung IRI, and whether PI3K/Akt pathway was involved in the protection effect of CB₂ receptors in lung IRI.

Materials and methods

Animals

Male C57BL/6 mice (Experimental Animal Center of Clinical Medical College of Sichuan University) aged 8 weeks were housed under controlled temperature (25±1°C), humidity (60± 10%), and light (12 h/day). All animal studies were in accordance with the Institutional Animal Care and Use Committee of Sichuan University (Chengdu, China, Approval No. 2018118A), and conformed to the guidelines of the National Institutes of Health (NIH), USA. CB₂ agonist JWH133 (259869-55-1) was purchased from Cayman Chemical (Michigan, MI, USA), and it was administered intraperitoneally (i.p.) to mice at 5 mg/kg. CB₂ antagonist AM630 (164178-33-0) was purchased from Sigma (St. Louis, MO, USA), injecting at a dose of 2 mg/kg. PI3K inhibitor LY294002 (S1105) was purchased from Selleckchem (Houston, TX, USA), injecting intraperitoneally at 5 mg/kg. The three drugs are dissolved in saline: Tween 80: dimethyl sulfoxide (DMSO) at a ratio of 18:1:1.

Lung ischemia reperfusion model

An in vivo mouse model of lung ischemic reperfusion was established based on our previously studies [15]. Mice were anesthetized by 1.0% pentobarbital sodium intraperitoneally (60 mg/kg). After endotracheal tube insertion, each mouse was ventilated with positive pressure using a rodent ventilator (Harvard Apparatus, Boston, MA) (FiO, 21%, 100 breaths/min, tidal volume of 8 ml/kg). Mice were placed in right lateral position, and the chest was opened by a left thoracotomy through the fourth intercostal space. Ischemia was induced by clamping the left pulmonary hilum with an artery clamp, and the ventilatory parameters were adjusted to a rate of 120 breaths/min and a tidal volume of 6 mL/kg. After 1 h of ischemia, the occlusion was released for 2 h sustained reperfusion, and ventilation was adjusted to a rate of 100 breaths/min and a tidal volume of 8 mL/kg. At the end of the experiment, the mice were euthanized with a large dose of pentobarbital sodium (100 mg/kg, i.p.).

Experimental protocol and group formation

In the first part, a total of 32 mice were randomly assigned into four groups to determine the role of activation of CB₂ receptors in Lung IR: (1) sham group: after thoracotomy, mechanical ventilation was carried out for 3 h without ligation of hilum. (2) Ischemic reperfusion (IR) group: after thoracotomy, left hilum was occluded for 1 h ischemia and then the occlusion was released for 2 h reperfusion. (3) JWH133+IR group: 5 min before occlusion, 5 mg/kg JWH133 was administered intraperitoneally, then the remaining steps were as same as IR group. (4) AM630+JWH133+IR group: AM630 was administered at a dose of 2 mg/kg 30 min before JWH133 infusion, then the remaining steps were as same as JWH133+IR group. In the second part, mice were designed to the following groups of sham, IR, JWH133+IR, and LY294002+JWH133+IR to determine the role of PI3K/Akt pathway. LY294002 was administered intraperitoneally at a dose of 5 mg/kg 30 min before JWH133 infusion, then the remaining steps were as same as in the JWH133+IR group.

Blood gas analysis

Arterial blood samples collected from the carotid arteries of the mice after reperfusion were analyzed by blood gas analyzer (Radiometer, Denmark). The ratio of PaO_2 to FiO_2 was obtained as an oxygenation index. The PaO_2/FiO_2 ratio is used to measure lung dysfunction.

Wet/dry weight ratio

The left lung was weighed to obtain the wet weight, and then placed in an oven at 60°C for 48 h and weighed until a stable dry weight was achieved. The wet/dry (W/D) weight ratio was calculated to assess tissue edema [16].

Histologic analysis

The left lung tissues obtained from all groups were processed and stained with hematoxylineosin. The histologic changes of left lung tissues were scored by a pathologist blind to the experimental group. The severity of the lung injury was scored based on alveolar edema, interstitial edema, hemorrhage, and inflammatory infiltration. The scoring system used in this work was based on previous studies [17, 18], where values from 0 to 3 represent the severity of the pathologic feature. The extent of lesions in each field of view was also evaluated based on the area the lesion covered, valued from O to 4. For each pathologic feature evaluated, the severity and the extent of involvement values were multiplied, leading to values in the range of 0-12.

Cytokine enzyme-linked immunosorbent assays for IL-6, TNF- α , MPO, MDA and SOD activity

The lung tissues were mixed with 4°C normal saline to obtain homogenate. Part of homogenate was centrifuged at 3000 rpm for 10 min at 4°C, and the supernatant (S1) was collected. Part of the homogenate was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatant (S2) was collected. Homogenate was used to measure the myeloperoxidase (MPO) and malondialdehyde (MDA) level of left lung, the supernatant (S1) was used to measure the superoxide dismutase (SOD) level, and the supernatant (S2) was used to measure IL-6 and TNF- α . MPO, MDA, SOD, IL-6 and TNF- α were measured by enzyme-linked immunosorbent assay (ELISA) kits (Nanjing Jiancheng, China; NeoBioscience, China) according to the manufacturer's instructions.

Western blot analysis

CB₂ receptor, Akt, and phosphorylated Akt (p-Akt) protein levels were assessed by western blot. The left lung was homogenized on ice in Radio-Immunoprecipitation Assay lysis buffer with protease/phosphatase inhibitor cocktail

(Cell Signaling Technology, MA) using a tissue grinder (Scientz, China). The homogenates were centrifuged at 12,000 rpm for 15 min at 4°C. Supernatants were collected, and the protein concentration was measured with a BCA protein assay kit using bovine serum albumin as the standard. SDS-PAGE protein electrophoresis was performed, and proteins were transferred to a PVDF membrane (Millipore, MA). The samples were blocked with 5% skim milk (Sigma, MO) in TBST for 2 h at room temperature. The membrane was rinsed with TBST and incubated with the primary anti-CB₂ (dilution 1:200; abcam, MA), anti-Akt (dilution 1:2000; Proteintech, IL) and anti-p-Akt (dilution 1:2000; Proteintech, IL) antibodies overnight at 4°C. The membrane was washed with TBST followed by incubation with corresponding secondary antibody for 2 h at room temperature. Bands were visualized using an ECL assay kit (Bio-Rad, CA) with Amersham-Imager 600 (General Electric Company, MA). The ratios of protein stripe gray value between target protein and β-actin protein provided a measurement of the CB_o receptor, Akt, and p-Akt protein levels.

Statistical analysis

SPSS 21 (SPSS Inc., Chicago, IL) was used for statistical analysis. The distribution of quantitative data was analyzed by the Kolmogorov-Smirnov test. Normally distributed variables were presented as mean ± standard deviation. Levene's test was used to evaluate the homogeneity of variances. The levels of PaO₂/FiO₂, left lung W/D ratio, SOD, MDA, MPO, TNF-α, IL-6, CB₂ protein, Akt and p-Akt protein were compared between the different treatment groups using one-way ANOVA. Multiple comparisons of differences between two groups were performed using the Tukey-HSD test or Tamhane's T2 test. The histological scores of lung injury were analyzed by nonparametric post hoc Kruskal-Wallis test.

Results

Activation of \mbox{CB}_2 receptors a meliorated lung IRI

Activation of CB_2 receptors increased PaO_2 / FiO₂, decreased lung W/D ratio and lung injury scores following IR: PaO_2 /FiO₂ ratio of IR group decreased significantly and the lung W/D ratio was significantly higher compared with the sham group (P<0.01). Administration of JWH133 Cannabinoid receptor-2 in lung reperfusion injury



Figure 1. Effects of the CB₂ agonist JWH133 on PaO₂ (A), lung W/D ratio (B), SOD activity (C), MDA content (D), TNF- α (E), IL-6 (F), MPO (G), and histologic changes (H) in mice after ischemia-reperfusion. (A) JWH133 reduced PaO₂, effects that were reversed by AM630. (B-G) JWH133 treatment improved lung injury as evidenced by a lower W/D ratio, MDA, TNF- α , IL-6, and MPO levels, and higher SOD activity. AM630 reversed these improvements. (H) Typical H & E-stained lung sections (×200) from each group. #P<0.05, ##P<0.01 compared with sham group; **P<0.01 compared with IR group; §P<0.05, §§P<0.01 compared with 133+IR group.

Group	Intra-alveolar edema	Interstitial edema	Hemorrhage	Inflammatory infiltrate
Sham	0.67±0.52	1.83±0.75	0.83±0.75	2.83±0.98
IR	1.83±0.75#	6.83±1.33 [#]	2.5±0.55#	8.17±1.17#
JWH133+IR	1.17±0.41	2.33±0.52*	1.67±0.52	4.17±0.98*
AM630+JWH133+IR	2.33±0.52 ^{#,§}	7.17±1.33 ^{#,§}	2.67±0.82#	7.5±1.22 ^{#,§}

 Table 1. Scores of histologic evaluation in four groups

*P<0.05, *P<0.05, §P<0.05.

before ischemia increased PaO_2/FiO_2 ratio and decreased W/D ratio significantly when compared with the IR group (P<0.01), but the PaO_2/FiO_2 ratio was lower and W/D ratio was higher in AM630+JWH133+IR group than in JWH133+ IR group (P<0.01), with no significant difference between the IR and AM630+JWH133+IR groups (**Figure 1A, 1B**).

Results of histologic evaluation are summarized in **Table 1**. The scores of intra-alveolar edema, interstitial edema, hemorrhage, and inflammatory infiltration in the IR group were higher than that in the sham group (P<0.05). Compared with the IR group, JWH133 pretreatment decreased the scores of interstitial edema and inflammatory infiltration (P<0.05). AM-630 increased the scores in three parts expect for hemorrhage compared with JWH133+IR group (P<0.05). Typical histologic features in the lung tissues are shown in **Figure 1H**.

Activation of CB_2 receptors decreased proinflammatory factor expression, increased SOD, and decreased MDA content following IR: As shown in **Figure 1E-G**, IR caused significant increases in the levels of TNF- α and IL-6 when compared with those of sham group (P<0.05). Addition of JWH133 before ischemia reduced the production of TNF- α and IL-6 after reperfusion as compared to the IR group, but pretreatment of AM630 before JWH133 increased the levels of TNF- α and IL-6 as compared to JWH-133+IR group (P<0.05). The change in MPO was similar to the change pattern of TNF- α and IL-6.

SOD is an antioxidant enzyme that protects cells from reactive oxygen species. As compared with the sham group, SOD activity was significantly reduced in the IR group (P<0.01). The SOD activity in the JWH133+IR group (P<0.01) was significantly increased compared with IR group, but AM630 (P<0.01) inhibited the effect of JWH133 (**Figure 1C**). MDA content, an index of lipid peroxidation, was significantly

increased in the IR group (P<0.01). JWH133 significantly attenuated the increase of MDA induced by IR, which was inhibited by pretreatment of AM630 (**Figure 1D**).

The role of PI3K/Akt in decreases of lung IRI induced by activation of CB₂ receptors

PI3K inhibitor worsened PaO₂/FiO₂ ratio and W/D weight ratio improved by JWH133: Administration of JWH133 before ischemia increased PaO₂/FiO₂ ratio and decreased the W/D ratio (IR vs. JWH133+IR, P<0.01), but the effect of JWH133 was abolished by pretreatment of LY294002 before JWH133 (IR vs. LY294002+JWH133+IR, P>0.05), with significant differences between JWH133+IR and LY294002+JWH133+IR groups (JWH133+IR vs. LY294002+JWH133+IR, P<0.01) (**Figure 2A, 2B**).

PI3K inhibitor elevated lung injury scores and proinflammatory factor expression, decreased SOD, and increased MDA content: Results of histologic evaluation are summarized in **Table 2**. Compared with the IR group, administration of JWH133 before ischemia decreased the scores of interstitial edema and inflammatory infiltration (P<0.05). Pretreatment of LY294002 increased the scores of intra-alveolar edema, interstitial edema and inflammatory infiltration compared with the JWH133+IR group (P<0.05), and did not show a significant difference compared with the IR group (P>0.05). Typical histologic features in the lung tissues are shown in **Figure 2H**.

As shown in **Figure 2C-G**, administration of JWH133 before ischemia decreased the level of TNF- α , IL-6, MPO activity and MDA content (IR vs. JWH133+IR, P<0.01), while SOD activity was significantly increased. The effect of JWH133 was abolished by pretreatment of LY294002 before JWH133 (IR vs. LY294002+JWH133+IR, P>0.05). The level of TNF- α , IL-6, MPO activity, and MDA content were signifi-

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Figure 2. Effects of the PI3K pathway on the PaO₂ (A), lung W/D ratio (B), SOD activity (C), MDA content (D), TNF- α (E), IL-6 (F), MPO (G), and histologic changes (H) in mice after ischemia-reperfusion. (A) JWH133 reduced PaO₂, effects that were reversed by LY294002. (B-G) JWH133 treatment improved lung injury as evidenced by lower W/D ratio, MDA, TNF- α , IL-6, and MPO levels, and higher SOD activity. LY294002 reversed these improvements. (H) Typical H & E-stained lung sections (×200) from four groups. #P<0.05, ##P<0.01 compared with sham group; **P<0.01 compared with IR group; §P<0.05, §§P<0.01 compared with 133+IR group.

Group	Intra-alveolar edema	Interstitial edema	Hemorrhage	Inflammatory infiltrate
Sham	0.63±0.52	1.87±0.64	0.88±0.64	2.88±0.99
IR	1.88±0.64##	6.88±1.25##	2.5±0.53**	8.25±1.03##
JWH133+IR	1.13±0.35*	2.38±0.52**	1.63±0.52*	4.25±1.16**
LY294002+JWH133+IR	2±0.53##,§	7.5±1.3##,§§	2.5±0.76 ^{##,§}	8±1.31##,§§

Table 2. Scores of histologic evaluation in four groups

^{##}P<0.01 compared with sham group. P<0.05, P<0.01 compared with IR group. P<0.05, P<0.01 compared with JWH133+IR group.



Figure 3. Expression of CB₂ receptor (A) and p-Akt (B) in four groups. ##P<0.01 compared with sham group. **P<0.01 compared with IR group. \$P<0.01 compared with JWH133+IR group.

cantly increased in the LY294002+JWH133+IR group as compared to the JWH133+IR group (P<0.01), while SOD activity was significantly decreased in LY294002+JWH133+IR group as compared to the JWH133+IR group (P<0.01).

JWH133 increased CB_2 receptor expression and PI3K inhibitor did not affect it: CB_2 expression in the IR group was a little bit higher than sham group, but there was no significant difference (sham vs. IR, P>0.05). JWH133 significantly increased CB_2 receptor expression in the lung tissues (JWH133+IR vs. IR, P<0.01). CB_2 receptor expression induced by JWH133 was not inhibited by pretreatment with LY29-4002 (JWH133+IR vs. LY2940024+JWH133+ IR, P>0.05) (Figure 3A).

Activation of CB_2 receptors increased p-Akt expression following IR: JWH133 was significantly increased in the expression of p-Akt when compared with the IR and sham group (P<0.01). In the LY294002+JWH133+IR group, the expression of p-Akt was decreased as compared to JWH133+IR group (P<0.01). Total Akt expression had no difference in all groups (**Figure 3B**).

Discussion

The present study revealed that activation of CB_2 receptors with JWH133 exerted pulmonary protective effects as evidenced by increased oxygenation index and ameliorated lung W/D ratio, decreased pro-inflammation cytokines, and MDA level, and elevated SOD activity. In contrast, the protective effect of CB_2 activation was abolished by pretreatment with CB_2 antagonist AM630. Pretreat-

ment with PI3K inhibitor LY294002 also weakened the CB₂ receptor-mediated protective effect. Additionally, activation of CB₂ receptors increased the expression of CB₂ receptors and p-Akt, but PI3K inhibitor only decreased the expression of p-Akt with no influence on CB₂ receptor.

In recent years, the endocannabinoid system has emerged as a new therapeutic target in ischemic reperfusion injury. It has been reported that pretreatment with URB602 (MAGL inhibitor) significantly reduced lung IRI and inflammation through increasing 2-AG level and reducing downstream metabolites [11], and 2-AG is a full agonist of the CB₂ receptor [19]. It is well known that JWH133 is a selective agonist of the CB₂ receptor [20], and AM630 is a selective antagonist of the CB₂ receptor [21]. The previous study had demonstrated that activating CB₂ by JWH133 reduced heart IRI [12]. Feizi et al. and Jeffrey et al. showed that CB₂ agonist reduced renal IR-induced lesions [22, 23]. Our study found that administration of CB_2 agonist JWH133 before IR increased PaO_2/FiO_2 ratio and decreased lung W/D ratio as well as lung injury scores, but injection of CB_2 antagonist AM630 before JWH133 abolished the effects of JWH133 on IR injured lung, which is in agreement with previous studies. JWH133 also significantly increased CB_2 receptor expression. These results indicated that activating of CB_2 receptors had a protective role against IR-induced lung injury in mice.

Oxidative stress is involved in pathogenesis of LIRI [3]. After pulmonary IR, oxygen free radicals are generated in lung tissues [24]. SOD is an antioxidant enzyme that protect cells from reactive oxygen species, reflecting the antioxidative capability. MDA, liberated as an end product from hydroxyperoxide destruction, is an indicator of lipid peroxidation. Wang et al. showed that CB₂ agonist markedly decreased the MDA level in infarcted heart [25], which is consistent with our results that activation of CB, receptor reduced lung MDA level and elevated SOD activity in LIRI and AM630 pretreatment before JWH133 presented reverted results. Therefore, it was suggested that CB₂ activation inhibited oxidative stress in lung IRI. Furthermore, lung IRI produces a large number of inflammatory substances and increases the permeability of pulmonary vascular endothelial cells and alveolar epithelial cells, leading to lung injury [26]. MPO is an enzyme secreted by activated leukocytes, and TNF-a and IL-6 are important inflammatory factors. In this experiment, levels of TNF- α , IL-6 and MPO were significantly elevated in left lung tissue of mice after lung IR. Activating CB2 receptors with JWH133 significantly reduced the levels of TNFα, IL-6, and MPO. However, treatment of AM630 before JWH133 significantly increased the levels of TNF-a, IL-6, and MPO. These findings indicate that CB, activation effectively inhibited IR-induced inflammation and ameliorated lung IRI. Our results are consistent with previous studies: activation of CB2 receptors decreased the expression of TNF- α and intercellular adhesion molecule-1, and reduced neutrophil infiltration, thereby leading to inflammation inhibition in the model of hepatic IR [27]. Javed et al. reported that CB₂ receptors activation improved the level of proinflammatory cytokines (IL-

1 β , IL-6, and TNF- α) in Rotenone Model of Parkinson's Disease [28].

However, it remains unclear that what is the specific mechanism of CB_2 activation in protecting IR injury. Li and colleagues found that activating CB_2 receptors reduced myocardium IRI by the PI3K/Akt pathway in a rat model [12]. Viscomi et al. reported that stimulation of CB_2 receptors generated a neuroprotective effect through PI3K/Akt pathway [29]. Li et al. demonstrated that AEA protected hearts against IRI through PI3K/Akt pathway, which was mediated by CB_2 rather than CB_1 receptors [30].

Consistent with these studies, we found that administration of JWH133 alleviated lung IRI and increased the p-Akt expression in injured lung, indicating that the PI3K/Akt pathway was involved in the protection of CB, activation in lung IRI. Interestingly, after activation of the CB₂ receptor, not only the expression of CB₂ receptor was significantly increased, but also the expression of p-Akt was significantly increased. In addition, the expression of CB₂ receptor was still at a high level after intervention with PI3K inhibitor LY294002, while the expression of p-Akt was significantly decreased. Therefore, this study showed that injection of LY294002 before IWH133 blocked the increase of p-Akt expression induced by JWH133. Similarly, pretreatment with LY294002 before JWH133 abolished the improvement induced by JWH133. These results further supported the idea that the protection of CB₂ activation against lung IRI depend partially on the PI3K/ Akt pathway.

Nevertheless, there are several limitations of the present study. Firstly, the CB_2 receptor depleted animals had not been used to confirm the role of CB_2 receptor in lung ischemia reperfusion injury. Another limitation is that the delayed protective effect of JWH133 on lung IRI was not explored. Additionally, whether activation of PI3K/Akt pathway can protect lung IRI independent of CB_2 receptor remains unknown. Further studies are still undergoing to explore the above issues.

Conclusion

Our study demonstrates that activation of CB_2 receptors plays a protective role against IR-induced lung injury through reducing inflammation in mice. PI3K/Akt pathway may be involved in the protective effect induced by activation of CB₂ receptors in LIRI.

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Disclosure of conflict of interest

None.

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