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Action of a Novel PDE4 inhibitor ZL-n-91 on lipopolysaccharide-induced acute lung injury

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ABSTRACT

In the present study, we investigated the effect of classic PDE4 inhibitor rolipram and novel PDE4 inhibitor ZL-n-91 on LPS-induced acute lung injury (ALI) in mice and its mechanism. ALI was induced in ICR mice by instilling intratracheally with LPS, and mice were divided into seven groups: control (Saline), LPS group, ZL-n-91 (3 μg, 10 μg, and 30 μg kg^{-1}, ip), Rolipram (1.0 mg kg^{-1}, ip) and dexamethasone (0.5 mg kg^{-1}, ip). After the 6 h of instilling intratracheally with LPS in mice, total leukocyte number, neutrophil number and protein content in BALF increased rapidly, a large number of neutrophil infiltration around the pulmonary vessel and airway, the lung wet weight/dry weight (w/d)ratio raised significantly. MPO activity, TNF-α level and cAMP-PDE, PDE4 activity in lung homogenate raised significantly. P_aO_2, P_aCO_2 and PH value in peripheral arterial blood also changed obviously, P_aO_2 and PH value dropped slightly and P_aCO_2 increased significantly in LPS group. ZL-n-91 (3 μg, 10 μg, 30 μg kg^{-1}) dose-dependently reduced the total leukocyte number, neutrophil number and total protein content in BALF, MPO activity, TNF-α level and cAMP-PDE, PDE4 activity in lung homogenate, but the effect of ZL-n-91 in pathological changes and lung wet w/d ratio is slight; Rol and Dex significantly reduced lung wet w/d ratio and improved pathological changes, neutrophil around the pulmonary vessel and airway significantly reduced, symptoms of lung edema relieved; The PH value, P_aO_2 and P_aCO_2 in ZL-n-91 high dosage group and Rol group had changes, but there was no significant difference compared with LPS group or saline group; After the administration, the righting reflex recovery time significantly shorten in every group of ZL-n-91. the righting reflex recovery time of Rol group was similar with ZL-n-91 30 μg kg^{-1} group, while Dex group was similar with saline group. The present study confirms that the inhibitory effect of ZL-n-91(30 μg kg^{-1}) on the inflammatory reactivity, including inhibition of inflammatory cell and protein exudation, MPO and PDE4 activity, improvement of the blood gas, those effects were equivalent with rolipram 1 mg kg^{-1}, and suggested that ZL-n-91 was stronger than rolipram in PDE4 inhibition. So we speculated that ZL-n-91 may have stronger therapeutic potential for treatment of inflammatory disease than rolipram, meantime have stronger nervous system effect than rolipram.

1. Introduction

Adenosine 3′, 5′-cyclic monophosphate (cAMP), 3′, 5′-cyclic guanosine monophosphate (cGMP) are the important second messengers of cells, which involved in the regulation of ALI/ARDS the pathogenesis by inflammatory cells and immune cells. cAMP and cGMP can combine with specific protein kinase leading to the phosphorylation of many substrates, regulating the various pathophysiological process. Phosphodiesterase (PDE) is the only way to the degradation of cyclic nucleotide and plays a key role to regulate such second messengers within cell level. cAMP can inhibit a variety functions of immune and inflammatory cells, in which cAMP-specific PDE4 is the major form of PDE, PDE4 inhibitors can increase the level of cAMP, and have shown anti-inflammatory effect in various animal models.

Selective inhibitors of PDE4 form the largest group among any PDE families, and have been studied as anti-inflammatory drugs for treatment of asthma and chronic obstructive pulmonary disease (COPD) and also as therapeutic agents against rheumatoid arthritis, multiple sclerosis, type II diabetes, septic shock, atopic dermatitis and other autoimmune diseases [4]. Almost all pharmaceutical giants such as Pfizer and GSK have been working on PDE4 inhibitors for treatment of asthma or COPD and more than 500 patents have been issued to PDE4 inhibitors internationally. However, no PDE4 inhibitors have been approved as the drugs because of their side effects such as emesis. Two best examples among the published PDE4 inhibitors are cilomilast and roflumilast. Cilomilast (Aryflo) was developed by SmithKlineBeach, now GlaxoSmithKline (GSK). Roflumilast was originally developed by

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Altana Pharma AG, Germany and later Pfizer and Tanabe joined in to look for approval in USA and Japan. These two drugs are in phase III clinical trial for treatment of asthma and COPD. However, roflumilast looks more potential.

Novel PDE4 inhibitor ZL-n-91 was gifted by Dr. Hengming Ke at University of North Carolina at Chapel Hill. The activity of ZL-n-91 shows an IC_{50} of 18 nM for inhibition on PDE4D2 and very active on inhibition of LPS-induced tumor necrosis factor α (TNFα) release in the human peripheral blood mononuclear cell (PBMC) system. TNFα is an inflammatory cytokine that activates neutrophils and mononuclear cells [2,13]. Lipopolysaccharide (LPS) is a key inflammatory stimulus that induces TNFα production and causes lung injury [2]. The inhibition of LPS-induced TNFα production is an essential assay for evaluation of the potency of PDE4 inhibitors in anti-inflammation and the treatment of COPD and asthma [6,7]. The IC_{50} values for inhibition of TNFα release were 0.04 and 0.06 μM for ZL-n-91 and rolipram (Rolipram was an anti-depressant in past, but now is discontinued). In comparison, another group has published that rolipram (IC_{50} = 1.3 μM) is about 8-fold more potent than cilomilast (IC_{50} > 10 μM, [7]). Thus, if rolipram is taken as a relative reference, the potency of ZL-n-91 is much better than that of cilomilast that is a drug lead in clinic trials for treatment of respiratory disease.

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), the development of which are associated with pneumonia, aspiration, sepsis, acute pancreatitis, drug overdose and so on are clinical syndromes associated with acute respiratory failure. It often showed as refractory hypoxemia, acute respiratory distress in clinical features, bilateral pulmonary infiltrates on chest radiograph, and often associated with multiple organ failure in later stage. There is no specific medicine to control ALI or ARDS as yet, so searching for the new therapy target and exploring the new drug to treat ARDS becomes the work of scientific research.

In this study, we want to discuss the action of a novel PDE4 inhibitor ZL-n-91 compared with the classical PDE4 inhibitor rolipram on LPS-induced acute lung injury in mice, and further explore its mechanism.

2. Materials and methods

2.1. Intratracheal LPS exposure

Acute lung injury was done as previously described, Briefly, male ICR mice weighing 20 ± 2 g, obtained from the animal center of Zhejiang University. Experimental protocols were approved by the Animal Care Committee of Zhejiang University in accordance with the international guidelines. Before the experiment, animals were fasted overnight and anesthetized with intraperitoneal 10% chloral hydrate 4 mg/kg. A tracheostomy was done and 3 mg/kg LPS was instilled. Sham-treated animals received the same volume saline only. Animals were maintained at 37 °C with until they recovered from the anesthesia. Six hours later, the mice were killed by a lethal injection of ethyl carbamate, and samples were collected.

2.2. Administration of Zl-n-91, rolipram and dexamethasone

Zl-n-91(3 μg/kg, 10 μg/kg, 30 μg/kg), rolipram (1.0 mg/kg) and dexamethasone (0.5 mg/kg) were intraperitoneal injected in 5 min after the LPS was administered.

2.3. Physiological measurements and blood gas

To confirm the efficacy of LPS intratracheal instillation, blood gas levels were determined at the end of the intratracheal LPS protocol. The mice were anesthetized with intraperitoneal 10% chloral hydrate 4 mg/kg, then carotid artery were separated by blunt dissection, using capillary column collected blood, arterial blood PH, P_aO_2 and P_aCO_2 were measured immediately by Blood Gas Analyzer (Radiometer ABL 700 Blood Gas Analyzer, Copenhagen, Denmark.).

2.4. Lung wet-to-dry weight ratios

Lungs were removed at the end of the experiment. The trachea and esophagus were separated from the lungs by blunt dissection, and the wet weight of the latter was determined. Subsequently, the lungs were incubated at 60 °C overnight to remove all moisture. The dry weight was then measured, and the ratio of wet-to-dry weight was calculated.

2.5. Bronchoalveolar lavage

Mice were anesthetized with intraperitoneal 10% chloral hydrate 4 mg/kg, then trachea was exposed and intubated with a 12-gauge cannula. The lungs were lavaged with 500 μl of PBS three times (total volume 1.5 ml). Fluid recovery was routinely ≥90%. The nucleated cells in BALF were counted in a hemocytometer as total leukocyte number. The BALF was immediately centrifuged at 500 × *g* for 10 min at 4 °C onto slides (700 *gav* for 4 min) and stained for 8 min with May and Giemsa stains. The slides were quantified for macrophages, neutrophils, and lymphocytes by counting a total of 200 cells/slide at 400 magnification.

2.6. Protein in bronchoalveolar lavage fluid (BALF)

The protein concentration in BALF was determined by the Bradford method using a protein assay kit (Nanjing Jiancheng Bioengineering Institute, China) with bovine serum albumin (Sigma) as a standard.

2.7. MPO activity and TNF-α assays

MPO activity and TNF-α concentration in 10% lung homogenized were determined using MPO activity kit (Nanjing Jiancheng Bioengineering Institute, China), and TNF-α ELISA kits (BD Bioscience, USA). All procedures were done according to the instructions of manufacturer.

2.8. Assay for cAMP-PDE and PDE4 activity

This experiment was conducted according to procedures described previously [12]. Briefly, frozen lungs were thawed and cut into small cubes, twenty-five mg of lung tissues was homogenized in 100 μl of ice-cold 30 mM HEPES (pH 7.4) containing 0.1% Trion X-100. cAMP-PDE assay mixture (200 μl) in PBS (pH 7.4) contained 137 mM NaCl, 2.7 mM KCl, 8.8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 μM cAMP (Sigma), and lung homogenate. The reaction was started by addition of 10 μl lung homogenate and performed at 37 °C for 10 min, then stopped by boiling the mixture for 3 min. The assay mixture was cooled on ice, followed by centrifugation at 12,000 × *g* for 30 min at 4 °C. The amount of cAMP present in the supernatant was determined by High performance liquid chromatography (HPLC) (Hypersil ODS 4.0 × 250 mm, Hewlett-Packard, Palo Alto, CA) using a standard curve of cAMP.

2.9. Morphologic assessment of lung injury

After intratracheal instillation of LPS 6 h, the mice were killed, and the lung lobe were collected and fixed with an intratracheal instillation of 1 ml formalin (pH 7.2, 4%) for 1 week. All slides were coded and evaluated in a blinded fashion to prevent bias. To evaluate the extent of lung injury, focus on the presence of any one of: *1*) capillary congestion; *2*) alveolar/interstitial edema; *3*) alveolar/interstitial hemorrhage; *4*) necrosis; and *5*) alveolar/interstitial neutrophils.

2.10. Assessing the anesthetic reversal effect of PDE4 inhibitors

Experiments were conducted following procedures previously described [9,10]. Briefly, mice were anesthetized with the combination of xylazine (10 mg/kg, sigma, USA) and ketamine (80 mg/kg, Zhejiang Xianju Pharmaceutical Co. LTD, China) administered in a single intraperitoneal injection. Fifteen minutes later, the animals were injected subcutaneously with various concentrations of test compound or vehicle and were subsequently placed in dorsal recumbency. All test compounds were freshly dissolved in 60% (vol/

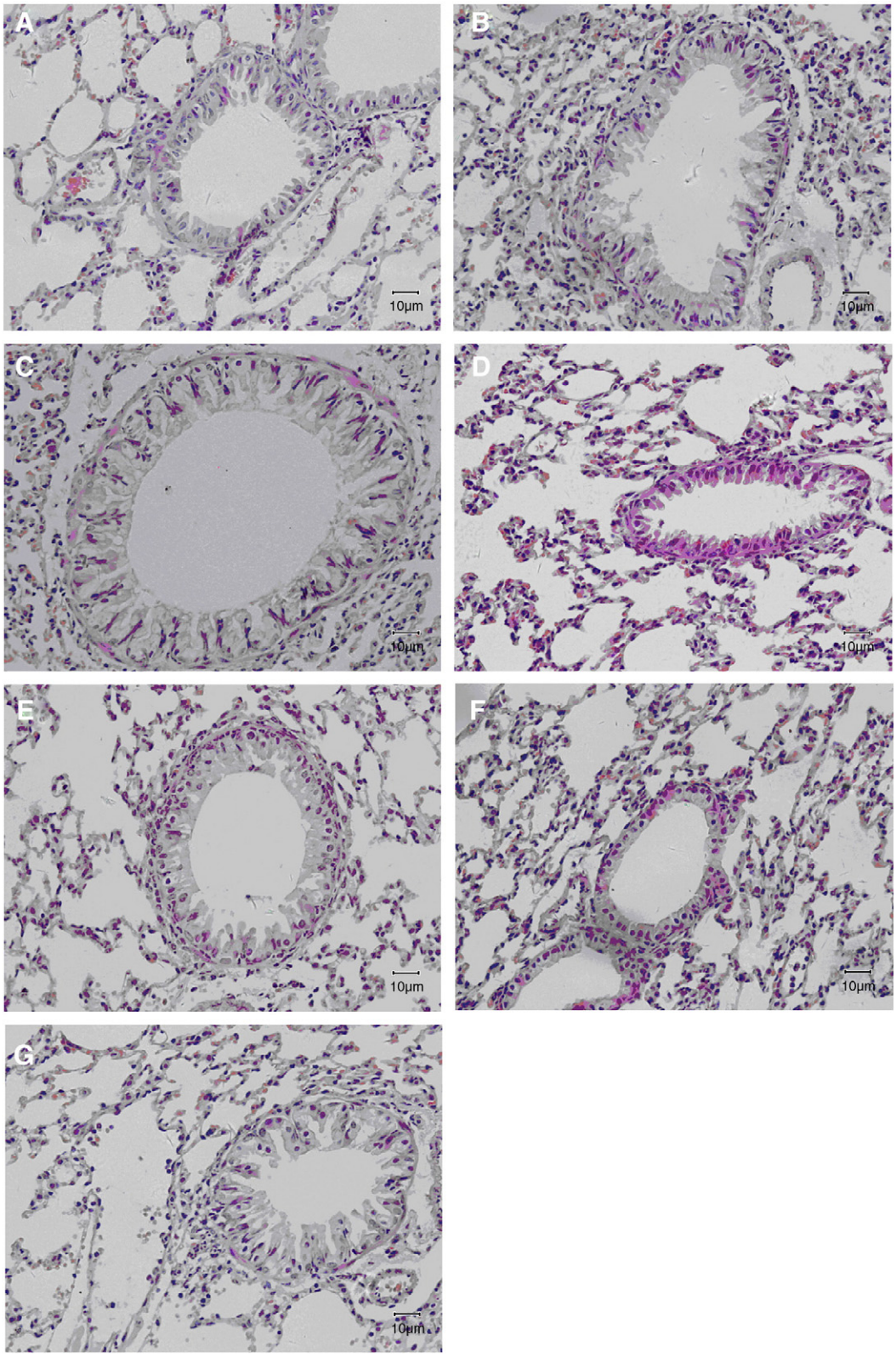

Fig. 1. Effect of ZL-n-91, Rol and Dex on lung histopathological changes in LPS-induced ALI mice (hematoxylin & eosin staining, 400× magnification). A: control; B: LPS; C: ZL-n-91 3 μg/kg; D: ZL-n-91 10 μg/kg; E: ZL-n-91 30 μg/kg ; F: Rol 1.0 mg/kg ; G: Dex 0.5 mg/kg.

vol) polyethylene glycol (PEG; molecular weight 200) in saline each day and administered in a dosing volume of 10 μl/g of body weight. The return of the righting reflex (i.e., when the animal no longer remained on its back and turned itself spontaneously to prone position) was used as an endpoint to measure the duration of anesthesia. For animals that had not restored their righting reflex in 120 min, we recorded the maximal amount of time allowed.

2.11. Statistical analysis

Results are reported as means ± SD. ANOVA was used to evaluate differences between groups. If significance was observed between groups, the Dunnet's *t*-test was used to compare the means of specific groups, with $P<0.05$ considered as significant.

3. Result

3.1. Zl-n-91 ameliorated LPS induced the changes in histology of lung

LPS was instilled intratracheally with or without PDE4 inhibitor or Dex treatment. Lung sections were prepared with hematoxylin and eosin (H&E) staining. A large number of neutrophil infiltration around the pulmonary vessel and airway were observed after LPS intratracheal instillation (Fig. 1B), while neutrophil infiltration seldom observed in saline controls (Fig. 1A). Pathological improvement was observed in all administrated groups in different degree, neutrophils around the pulmonary vessel and airway were reduced in all test groups, there were significantly relieved in ZL-n-91 30 μg kg^{-1}, Dex group and Rol group.

3.2. Zl-n-91 not obviously improved LPS-induced blood gas changes in ICR mice

Fig. 2-A and B summarizes the values for blood gases in mice after intratracheal instillation of LPS and intraperitoneal administration of test compounds. After the 6 h of instilling intratracheally with LPS in mice, P_aO_2, P_aCO_2 and PH value in peripheral arterial blood changed obviously, the PH value and P_aO_2 dropped significantly compared with control group, but had no statistically difference. P_aCO_2 in LPS group increased significantly compared with control group and had statistically difference ($P<0.01$). The PH value in ZL-n-91 group, Rol and Dex group was obviously decreased, compared with LPS group, there was no significant difference, but compared with saline group there were significant differences ($P<0.05$). PDE4 inhibitors could improve PaO_2 changes, especially in ZL-n-91 high dosage and Rol group, but compared with the LPS group and control group, there was no significant difference, P_aCO_2 in ZL-n-91 3 μg/kg, 10 μg/kg groups had not obviously improved, in ZL-n-91 30 μg/kg and Rol group, the P_aCO_2 improved significantly, but compared with LPS group and control group, there was no significant difference. Dex had not obviously influence on P_aO_2 and P_aCO_2.

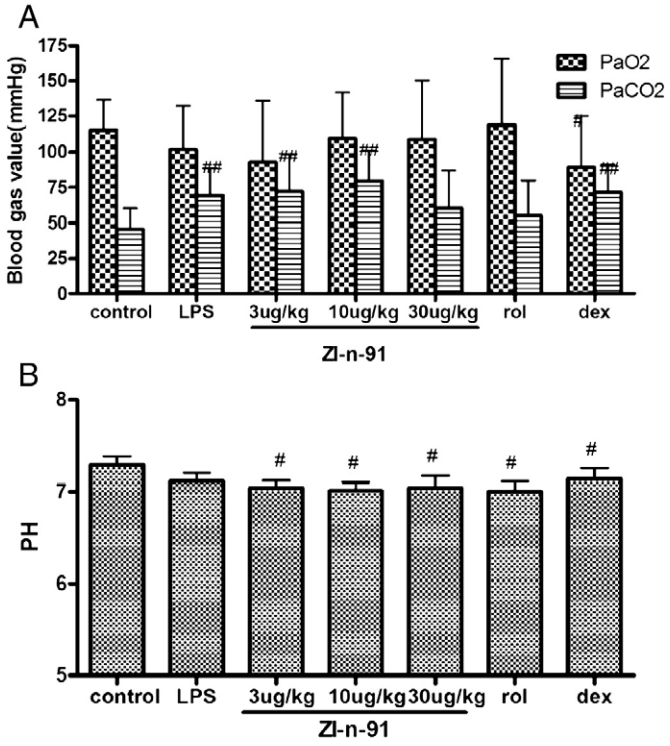

Fig. 2. Blood gas changes of ZL-n-91, rol and dex on LPS-induced ALI. (Data were expressed as mean ± SD, $n=7$–20 $^{\#\#}p<0.01$ *vs* control group, $^{*}p<0.05$, $^{**}p<0.01$ *vs* LPS group) . A: P_aO_2, P_aCO_2; B: PH value.

3.3. Zl-n-91 inhibited LPS-induced the cell and protein exudation in BALF

After the 6 h of instilling intratracheally with LPS, in LPS group, inflammatory cell number and neutrophil number in BALF raised rapidly by 2.86 times and 6.16 times, and protein content in BALF increased significantly by 1.35 times compared with the control group ($P<0.01$). ZL-n-91 (3 μg, 10 μg, 30 μg/kg) could dose-dependently reduce the total leukocyte cell number and neutrophil number in BALF ($P<0.01$) (Fig. 3A); Total protein content in BALF was significantly reduced in every ZL-n-91 group, 3 μg/kg had significant difference ($P<0.05$) (Fig. 3B). After Rol and Dex treatment, total leukocyte cell number, neutrophil number and total protein content in BALF were also obviously decreased ($P<0.01$), was equal to ZL-n-91 30 μg/kg group.

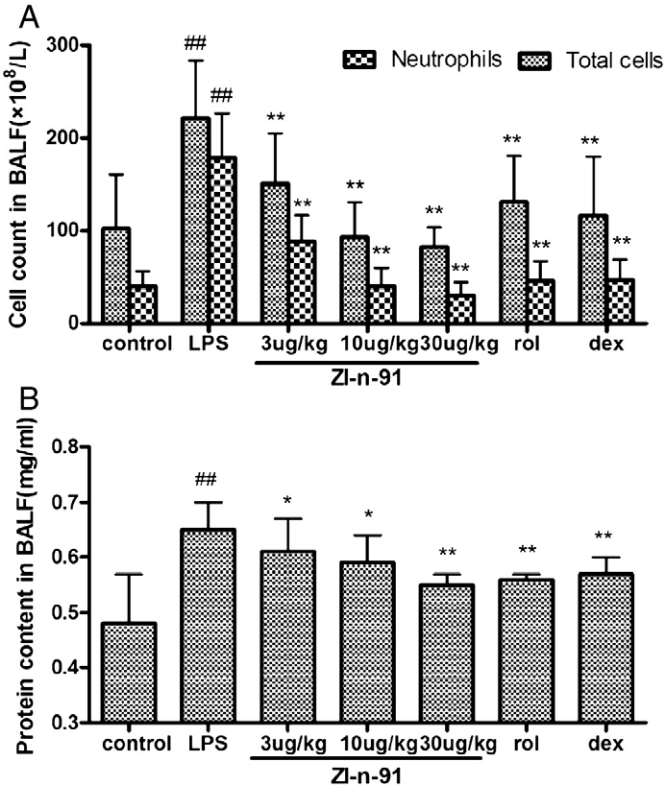

Fig. 3. The effect of ZL-n-91, rol and dex on the cell number and protein content of BALF in LPS-induced ALI mice . (Data were expressed as mean ± SD, $n=8$–12 $^{\#\#}p<0.01$ *vs* control group, $^{*}p<0.05$, $^{**}p<0.01$ *vs* LPS group) A:total leukocyte number and neutrophil number in BALF; B: protein content.

3.4. Zl-n-91 slightly inhibited LPS-induced the change of lung W/D ratio

The lung W/D ratio of LPS group raised significantly compared with saline group ($P<0.01$), Rol and Dex could significantly reduce lung W/D ratio ($P<0.05$), but in ZL-n-91 groups there were no significant difference compared with LPS group (Fig. 4).

3.5. Zl-n-91 significantly inhibited LPS-induced the changes of MPO activity, TNF-α level, cAMP-PDE and PDE4 activity in lung homogenate

In LPS group, MPO activity and TNFα-level in lung homogenate were increased sharply by 1.5 times and 2.2 times, cAMP-PDE and PDE4 activity in lung homogenate were raised significantly by 2.77 times and 1.95 times compared with the control group ($P<0.01$). Strong inhibition was observed in all test group. Zl-n-91 3 μg/kg, 10 μg/kg, 30 μg/kg dose-dependently reduced MPO activity (Fig. 5A), and significantly reduced TNF-α level (Fig. 5B); ZL-n-91 also obviously inhibited cAMP-PDE and PDE4 activity in lung homogenate compared with LPS group, ZL-n-91 10 μg/kg had remarkable difference ($P<0.01$)(Fig. 5C), and stronger than classical PDE4 inhibitor Rol and Dex. The effects of Rol and Dex on MPO activity, cAMP-PDE activity and PDE4 activity were similar with ZL-n-91, but Rol showed a stronger action on TNF-α secretion.

3.6. Zl-n-91have strong anesthetic reversal effect (Fig. 6)

Interestingly, the ability of PDE4 inhibitors to reverse anesthesia appears to reflect the emetic potential of these agents [8,15] (Fig. 6). Therefore, assessing the anesthetic reversal effect of PDE4 inhibitors could be an efficient way to evaluate the emetic potential of this class of compounds in rats, since rodents do not have a vomiting reflex. The recovery time of righting reflex in solvent group and saline group, there were no significant difference. After the administration, the righting reflex recovery time significantly shorten in ZL-n-91 every group, 3 μg/kg group compared with control group, there were no significant difference, but 10 μg/kg and 30 μg/kg group showed significant difference compared with vector group ($P<0.01$). Rol group was equivalent with high-dose group, while the Dex and saline control group were quite.

4. Discussion

For its best characteristic of special selectivity on PDE4D, novel PDE4 inhibitor Zl-n-91 has attracted attention of many researchers. One report has suggested that Zl-n-91 has direct inhibition of IL-17 production from memory Th17 cells [5]. Our previous report also suggested Zl-n-91 could decrease inflammation and improve lung function in COPD-like rat model prepared by challenging with LPS and cigarette smoking in male Sprague–Dawley rats, the mechanism maybe involve in inhibiting PDE4 activity and MMP-9 level in lungs [14]. Those study suggested Zl-n-91 have powerful potential as an alternative therapy for pulmonary diseases and regulation of immunity.

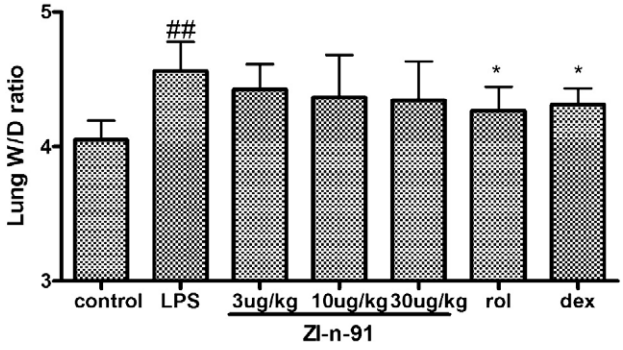

Fig. 4. The effect of ZL-n-91, rol and dex on lung W/D ratio in LPS-induced ALI mice . (Data were expressed as mean ± SD, $n=8$–12 $^{\#\#}p<0.01$ *vs* control group, $^{*}p<0.05$, $^{**}p<0.01$ *vs* LPS group).

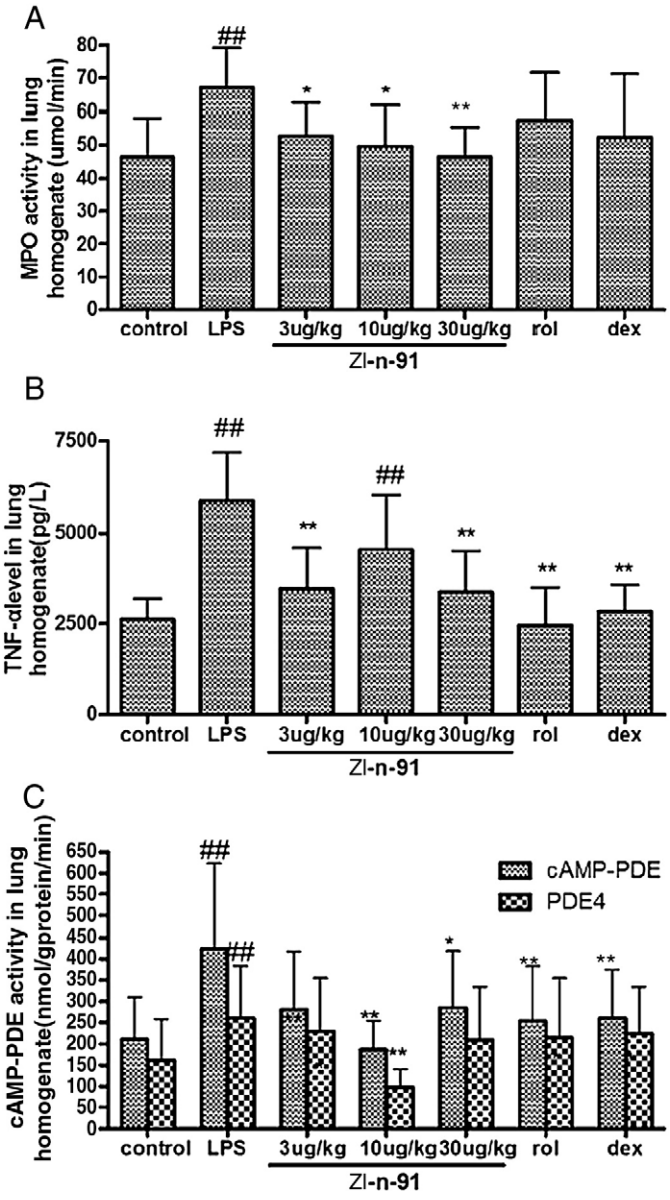

Fig. 5. The effect of ZL-n-91, rol and dex on MPO activity, TNF-α level, cAMP-PDE and PDE4 activity in lung homogenate in LPS-induced ALI mice . (Data were expressed as mean ± SD, $^{\#\#}p<0.01$ *vs* control group, $^{*}p<0.05$, $^{**}p<0.01$ *vs* LPS group) A:MPO activity ($n=8$–12); B: TNF-α level ($n=8$–12); C: cAMP-PDE and PDE4 activity ($n=8$–20).

In our present study, we used rolipram as a relative reference, the potency of our ZL-n-91 may be better than classical PDE4 inhibitors, in very lower concentration, ZL-n-91 (3 μg, 10 μg, 30 μg/kg) dose-dependently reduced the total leukocyte cell number and neutrophil number in BALF ($P<0.05$); In each dose group of ZL-n-91, the lung W/D ratios were reduced, but compared with the LPS group, there was no significant difference, while Rol and Dex significantly reduced lung W/D ratio ($P<0.05$) suggested that if we increase the dose of ZL-n-91, maybe we obtain significant results; pathological changes was improved in Rol groups, neutrophil around the pulmonary vessel and airway significantly reduced, symptoms of lung edema relieved, surprisingly, ZL-n-91 30 μg/kg also have similar effect; some parameters, such as protein content in BALF, MPO activity in lung tissues, cAMP-PDE and PDE4 activity in lung homogenate also significantly reduced in every ZL-n-91

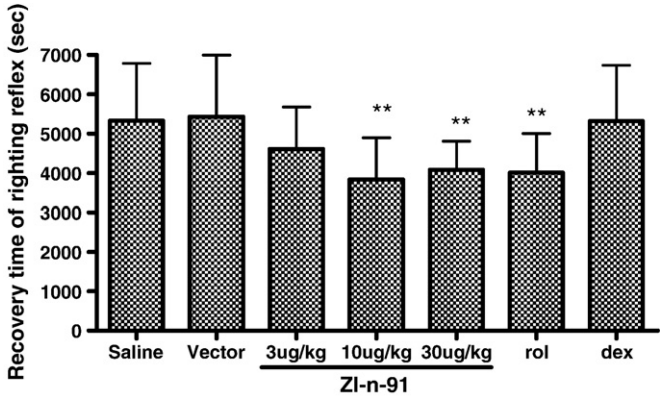

Fig. 6. Effect of ZL-n-91, rol and dex on the duration of anesthesia induced by the combination of xylazine (10 mg/kg) and ketamine (80 mg/kg). The duration of anesthesia was assessed by the return of the righting reflex. (Data were expressed as mean ± SD, $n = 25–30$. $^{**}p < 0.01$ *vs* saline or vector group).

group, high dosage Zl-n-91 was similar with rolipram, this result suggested that novel PDE4 inhibitor Zl-n-91 may be about 100-fold more potent than rolipram *in vivo*, it was an exciting result. Meanwhile the extent of ZL-n-91 inhibit the TNF-α secretion and improve blood gas aspect was little weaker than rolipram, previous data suggested that the IC_{50} values for inhibition of TNFα release in the human peripheral blood mononuclear cell (PBMC) system were 0.04 and 0.06 μM for ZL-n-91 and rolipram, because the low dosage of Zl-n-91 in this study, we speculated that increase the dosage of Zl-n-91 may improve the effect. As we know, rolipram was a non-selective PDE4 inhibitor, while Zl-n-91 was a PDE4D subtype-selective inhibitor, represented a new generation of PDE4 inhibitor. The different characteristic between Zl-n-91 and rolipram, suggested the further direction of our work.

Interestingly, corticosteroid drug dexamethasone also attenuated cAMP-PDE activity and PDE4 activity. Although it is not clear exactly how dexamethasone attenuated the PDE4 enzyme activity upregulation, one possibility was that they might attenuate PDE4 gene expression and/or activation through their extensive anti-inflammatory effects. For instance, corticosteroid drugs inhibit production of the various cytokines, which could result in the downregulation of PDE4 activity observed in this study. Dexamethasone also might directly inhibit PDE4 gene expression as suggested by a report [1].

Emesis is a major side effect associated with inhibitors of PDE4. The PDE4 family is composed of four subtypes (PDE4A, 4B, 4C, and 4D) and multiple splice variants. If it were possible to identify the subtype (s) responsible for the beneficial and the side effects associated with PDE4 inhibition, then subtype-selective inhibitors devoid of the tendency to induce nausea and vomiting could be developed. The mechanism of the emetic response associated with PDE4 inhibitors is thought to be a consequence of the inhibition of PDE4D subtype [9,10], But which PDE4D subtype was involved in the nausea action was unclear. Recently advance suggested that PDE4D modify the stroke risk in the Swedish population [3]. However, subsequent genetic case/control studies in other populations failed to confirm the association between PDE4D and stroke [11].We used a novel way of evaluating the emetic potential of PDE4 inhibitor in species that do not have a vomiting reflex (e.g., rodents). The observations made in mice indicate that our novel PDE4 inhibitor Zl-n-91 may have a stronger action than rolipram in reversing α2-adrenoceptor-mediated anesthesia, a behavioral correlate of PDE4 inhibitor-induced emesis in non-vomiting species [8–10]. A previous study had unveiled that the activity of ZL-n-91 shows an IC_{50} of 18 nM for inhibition on PDE4D2, which was due to the stronger nervous action than rolipram, for given evidence suggested rolipram was non-selective PDE4 inhibitor. So, if modification the formulation of Zl-n-91 to limiting central nervous system penetration, novel PDE4 inhibitor Zl-n-91 maybe an excellent anti-inflammatory drug for inflammatory disease.

In summary, using *in vivo* mouse models of ALI, the current study find some evidence to support the novel PDE4 inhibitor Zl-n-91 is beneficial in the treatment of ALI/ARDS. Considering the potential side effects of Zl-n-91, our results suggest that further studies are needed to clarify any potential role of Zl-n-91 in modifying the inflammatory progression.

Acknowledgments

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References

[1] Ahlstrom M, Pekkinen M, Huttunen M, Lamberg-Allardt C. Dexamethasone down-regulates cAMP-phosphodiesterase in human osteosarcoma cells. Biochem Pharmacol 2005;69:267–75.
[2] Barnes PJ. Chronic obstructive pulmonary disease[J]. N Engl J Med Jul 27 2000;343(4): 269–80 Review.
[3] Hugh S. Markus and Mark J. Alberts. Update on Genetics of Stroke and Cerebrovascular Disease. Stroke, 2005, Feb 2006; 37: 288–290.
[4] Lipworth BJ. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease[J]. Lancet 2005;365(9454):167–75.
[5] Ma R, Yang BY, Wu CY. A selective phosphodiesterase 4 (PDE4) inhibitor Zl-n-91 suppresses IL-17 production by human memory Th17 cells. Int Immunopharmacol [J] Oct 2008;8(10):1408–17.
[6] Muise ES, Chute LC, Claveau David, et al. (PDE4)selective inhibitors[J]. Biochem Pharmacol 2002;63:1527–35.
[7] Ouagued M, Martin C, Brinchault G, et al. The novel phosphodiesterase4 inhibitor. CI-1044.inhibits LPS-induced TNF-α production in whole blood from COPD patients[J]. Pulmonary Pharmacol Ther 2005;18(1):49–54.
[8] Robichaud A, Savoie C, Stamatiou PB, et al. PDE4 inhibitors induce emesis in ferrets via a noradrenergic pathway[J]. Neuropharmacology 2001;40(2):262–9.
[9] Robichaud A, Stamatiou PB, Jin SL, Lachance N, MacDonald D, Laliberté F, et al. Deletion of phosphodiesterase 4D in mice shortens alpha(2)-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis[J]. J Clin Invest Oct 2002;110(7):1045–52.
[10] Robichaud A, Savoie C, Stamatiou PB, Lachance N, Jolicoeur P, Rasori R, et al. Assessing the emetic potential of PDE4 inhibitors in rats[J]. Br J Pharmacol Jan 2002;135(1):113–8.
[11] Bevan Steve, Dichgans Martin, Gschwendtner Andreas, Kuhlenbäumer Gregor, Ringelstein EB, Markus Hugh S. Variation in the PDE4D gene and ischemic stroke risk: a systematic review and meta-analysis on 5200 cases and 6600 controls. Stroke Jul 2008;39:1966–71.
[12] Tang HF, Song YH, Chen JC, et al. Upregulation of phosphodiesterase-4 in the lung of allergic rats[J]. Am J Respire Crit Care Med 2005;171(8):823–8.
[13] Vassalli P. The pathophysiology of tumor necrosis factors[J]. Annu Rev Immunol 1992;10:411–52.
[14] Wang YJ, Jiang YL, Tang HF, Zhao CZ, Chen JQ. Zl-n-91, a selective phosphodiesterase 4 inhibitor, suppresses inflammatory response in a COPD-like rat model. Int Immunopharmacol 2009;10(2).
[15] Robichaud A, Tattersall FD, Choudhury I, Rodger IW. Emesis induced by inhibitors of type IV cyclic nucleotide phosphodiesterase (PDE IV) in the ferret. Neuropharmacology 1999 Feb;38(2):289–97.