

Original Article

A γ -Secretase inhibitor (DAPT) alleviates the inflammation of paraquat-induced ALI mice by regulating the differentiation of Th17 cells

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Received June 24, 2018; Accepted December 11, 2018; Epub April 15, 2019; Published April 30, 2019

Abstract: The Notch signaling pathway is critically involved in the proliferation and differentiation of T cells, key players in the pathogenesis of the inflammatory diseases. It was reported that Notch signaling can induce the differentiation of T helper 17 (Th17) cells and promote the expansion of interleukin-17 (IL-17). γ -Secretase inhibitors (DAPT) have been used to effectively block Notch signaling and directly regulate Th17 responses through a Notch signaling-dependent pathway. The aim of this study was to determine whether a γ -secretase inhibitor (DAPT) inhibits the differentiation of Th17 cells in mice with paraquat (PQ)-induced acute lung injury (ALI). DAPT ameliorated the inflammatory reaction of PQ-induced ALI. DAPT also significantly regulated Th17 cell responses and reduced the levels of IL-17A. These results suggested that DAPT could inhibit the differentiation of Th17 cells in mice with PQ-induced ALI. Thus, DAPT plays critical role in attenuating PQ-induced ALI and should be considered as a potential therapeutic agent.

Keywords: Paraquat, acute lung injury, Th17 cells, γ -secretase inhibitor

Introduction

Paraquat (PQ) is an organic heterocyclic herbicide, which is used worldwide in agriculture because of its effective control of weeds. However, previously published studies have shown that PQ is a highly toxic compound for both humans and animals, with a high mortality of approximately 60% [1, 2]. Most importantly, PQ selectively accumulates in the lungs which eventually leads to the development of acute lung injury (ALI) and irreversible pulmonary fibrosis [3]. Although the mechanisms of PQ-induced ALI are not fully elucidated, studies have provided data to support the hypothesis that inflammation and inflammatory mediators are associated with the occurrence and development of PQ-induced ALI [4].

Accumulating evidence has shown that CD4⁺ T cells play a pivotal role in the pathogenesis of ALI, especially T helper 17 (Th17) cells [5, 6]. Meanwhile, several studies have found that the Notch signaling pathway is critically involved in

the proliferation and differentiation of T cells, key players in the pathogenesis of the inflammatory diseases [7, 8]. Furthermore, Notch signaling can induce the differentiation of Th17 cells and promote the expansion of interleukin-17 (IL-17) in the pathogenesis of several inflammatory diseases [9, 10]. However, there are still no reports determining whether the Notch signaling pathway could cause changes in PQ-induced ALI. Moreover, evidence is lacking which shows the relationship between the Th17 response through a Notch signaling-dependent pathway and the pathogenesis of PQ-induced ALI. Accordingly, we hypothesized that regulating the Th17 response may have potential therapeutic effects in PQ-induced ALI.

As an effective blocker of Notch signaling, γ -secretase inhibitors (DAPT) play a crucial role in regulating the differentiation of Th17 cells [11]. Therefore, the aim of this study was to evaluate whether DAPT alleviated inflammation in mice with PQ-induced ALI by regulating the differentiation of Th17 cells, thus elucidating

whether the changes in Th17 cells in the PQ-induced mice are associated with the alteration of Notch expression.

Materials and methods

Experimental animals and protocols

Male Balb/c mice (6~8 weeks old, 18~22 g body weight) which were maintained under specific pathogen-free regions were obtained from the Laboratory Animal Center of Guangxi Medical University (Nanning, Guangxi, China). Room temperature and humidity were set at $23\pm 3^{\circ}\text{C}$ and $55.5\pm 10\%$, respectively. All mice were provided food and water *ad libitum* with a standard laboratory diet. Animal experimental protocols were consistent with the Ethical Principles in Animal Research adopted by the Guangxi Medical University for Animal Experimentation.

Establishment of mouse models

Experimental animals were randomly divided into three groups: the control group, the PQ group, and the DAPT group, with 6 mice in each group. According to our previous research and related reports [12], after measurement of their body weight, the mice received one injection of 20 mg/kg PQ in normal saline (Sigma, St. Louis, MO, USA) intraperitoneally. One group was treated with dimethylsulfoxide (DMSO) as a control for DAPT, while the control group was treated with normal saline instead of PQ at volumes equivalent to the PQ dosage. The mice in the DAPT group were injected with DAPT (40 mg/kg, re-suspended in DMSO) 30 minutes after PQ administration.

Mouse sample collection and processing

Twenty-four hours after treatment, all animals were anesthetized with pentobarbital. Specimens (BALF, lung, and spleen) were harvested. Bronchoalveolar lavage fluid (BALF) was collected as previously described [13]. Briefly, the mice were anesthetized by and intraperitoneal injection of 10% chloral hydrate and fixed on their backs. The lungs were lavaged three times using 500 μL of ice-cold PBS, and then the BALF was collected in Eppendorf tubes, on ice. The recovery of the BALF was more than 80%. The supernatant was separated by centrifugation at $1500\times g$ for 10 min at 4°C , aliquoted and then stored at -80°C prior to the measurement of cytokine levels.

To obtain splenocyte suspensions, the spleens were removed and cut into small pieces and subsequently ground gently into single cells and filtered through nylon mesh. The cell suspension was centrifuged at $300\times g$ for 10 min at 4°C . Subsequently, the erythrocytes were removed, as described previously, and the cell pellets of the spleens were washed twice with cold PBS.

The left upper lung tissues were fixed with 4% paraformaldehyde for H&E staining, the left lower lung tissues were used to analyze the wet/dry (W/D) weight ratios, and the right lung was snap-frozen by immersion in liquid nitrogen, and then stored at -80°C until quantitative real-time PCR was performed.

Histology and morphometry assay

The left upper lung lobes were isolated from the mice, fixed with 4% paraformaldehyde, embedded in paraffin wax, and sectioned in at 4 μm onto glass slides. The tissue sections were stained with hematoxylin and eosin (HE) for examination using an Olympus lighted microscope (Olympus, Tokyo, Japan), with the histological assessment performed with the reviewer being blinded to the experimental group in each case. The severity of lung tissue injury, hemorrhage, and inflammation was assessed.

Lung wet/dry (W/D) weight ratio

The lung W/D ratio was used to quantify the magnitude of pulmonary edema and the severity of lung injury. The left lower lung tissues were harvested at 24 h after treatments in all the groups of mice, weighed immediately, and then placed in an oven at 70°C for 72 h to obtain the dry weight. The W/D ratio was calculated using the following formula: The W/D ratio = the wet weight / the dry weight.

Cytokine measurement

A commercial enzyme-linked immunosorbent assay (ELISA) kit (Cusabio Biotech Co. Ltd, Wuhan, China) was used, according to the manufacturer's instructions to measure IL-17A in the BALF fluid for each of the study animals. The absorbance was measured at 450 nm by a microplate ELISA reader (Bio-Rad Laboratories, CA, USA).

Flow cytometry

The expression markers on the T cells from the spleens were observed by flow cytometry through the following antibodies: percp-CD4 and PE-IL-17, which were purchased from BD Pharmingen (San Diego, CA, USA) or eBioscience (San Diego, CA, USA). Cell surface staining was performed according to the standard procedures. For intracellular detection of cytokines, cells were stimulated with ionomycin (1 ng/ml; Sigma-Aldrich) and phorbol-myristate-acetate (PMA, 25 ng/ml; Sigma-Aldrich) in the presence of GolgiPlug™ (BD Pharmingen) for 4 h at 37°C in 5% CO₂. Then the cells were washed and stained with fluorescent antibodies against CD3 at room temperature in the dark. After surface staining, the cells were permeabilized in a permeabilization solution (Cytofix/Cytoperm™; BD Pharmingen), then stained with anti-IL-17 mAbs for 30 min at 4°C. The cells were washed with Perm/Wash Buffer (BD Pharmingen) and resuspended in PBS + 2% FBS for flow cytometric analysis. Flow cytometry was determined on a BD FACS Canto II (BD Biosciences) and analyzed with FCS Express 4 software (De Novo Software, Los Angeles, CA, USA).

Quantitative real-time polymerase chain reaction (PCR) analysis of RORγt mRNA expression

To quantify the Th17 transcription factor retinoid-related orphan nuclear receptor (RORγt), total RNA was extracted from lung samples with TRIzol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. cDNA was prepared using an oligo (dT) primers (PrimeScript™RT reagent Kit, TAKALA, Kusatsu, Japan). Quantitative RT-PCR was performed in duplicate with SYBR Green I (SYBR®Premix Ex Taq™, TAKALA) using an Applied Biosystems 7500 analyzer (ThermoFisher Scientific) according to the manufacturer's instructions. DNA was amplified under the following conditions: denaturation at 95°C for 30 s, extension at 95°C for 5 s, 60°C for 34 s, and the samples were amplified for 40 cycles. The following primers were used: 5'-AATCCATCATGAAGTGTGA-3', 5'-ACTCCTGCTTGCTGATCCAC-3' for β-actin; 5'-TGCC AGGAGTGAACCTGGAC-3' and 5'-CCGAAGTGTGGTAGC GAGGA-3' for RORγt. β-actin was used as an internal control, and the levels of each gene were normalized to β-actin expression using the ΔΔCt-method. The identity of the amplified products was exam-

ined using agarose gel electrophoresis and melt curve analysis.

Statistical analysis

The statistical analysis of data was performed using the SPSS statistical software (version 19.0, SPSS IBM, Chicago, IL, USA) and GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). The data are presented as the means ± standard deviation (SD). Statistical comparisons of data were performed using a one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test of means. *P*-values of less than 0.05 were considered to indicate statistically significant differences.

Results*The inhibitory effects of DAPT ameliorate the severity of inflammation in PQ-induced ALI*

Histological changes in the lungs of the mice in each group were evaluated by light microscopy of the H&E-stained tissue sections. The representative sections from the lungs of the mice in each experimental group are shown in **Figure 1**. The control group showed normal lung tissue structure, and no inflammation and destructive changes. The PQ-treated mice showed dilatation of the alveolar capillaries, which were filled with blood, and acute inflammatory cell infiltrates in the alveolar spaces, consisting mainly of neutrophils, with lung injury. Compared with the PQ group, the DAPT group showed markedly attenuated inflammatory cell infiltration.

Effect of DAPT on mouse wet to dry (W/D) lung weight ratios

In this study, the effects of DAPT on PQ-induced lung wet to dry (W/D) weight ratios were assessed. The W/D lung weight ratios were measured to detect the magnitude of the pulmonary edema and the severity of lung injury. As shown in **Figure 2**, compared with the control group, W/D ratios were significantly increased in the PQ group. Interestingly, treatment of DAPT markedly decreased the lung W/D weight ratios in the DAPT group.

DAPT treatment regulates the production of IL-17A in PQ mice

Inflammatory cytokines play a key role in the pathogenesis of PQ-induced ALI. To evaluate

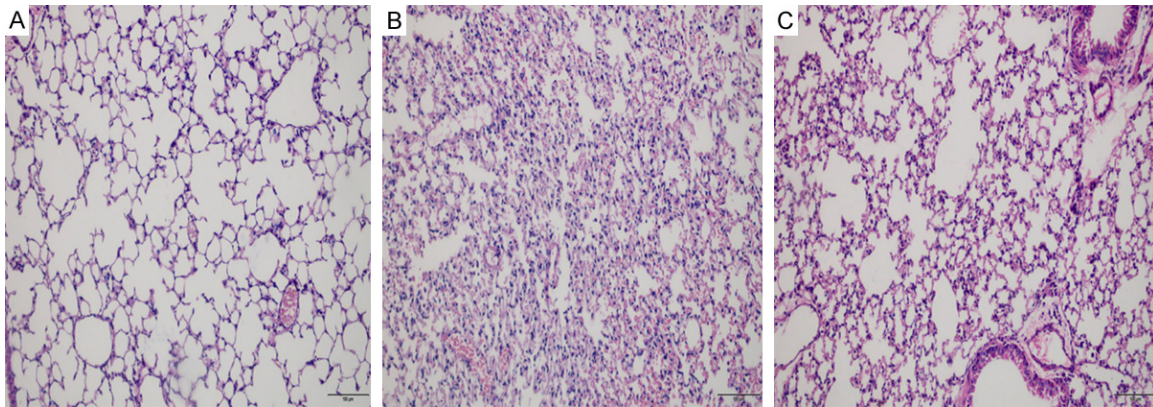


Figure 1. Histological changes of lung tissues by H&E staining. (A) Photomicrograph of the histology of a tissue section from the control group shows the normal lung tissue structure, with no inflammation; (B) Histology of a tissue section of lung from the PQ group shows dilatation of the alveolar capillaries, which are filled with blood. There are also acute inflammatory cells in the alveolar spaces, consisting mainly of neutrophils, and lung injury is present. (C) Histology of a tissue section of lung from the DAPT group showed significantly alleviated inflammatory cell infiltration. (A) Control group, (B) PQ group, (C) DAPT group. Original magnification: $\times 200$.

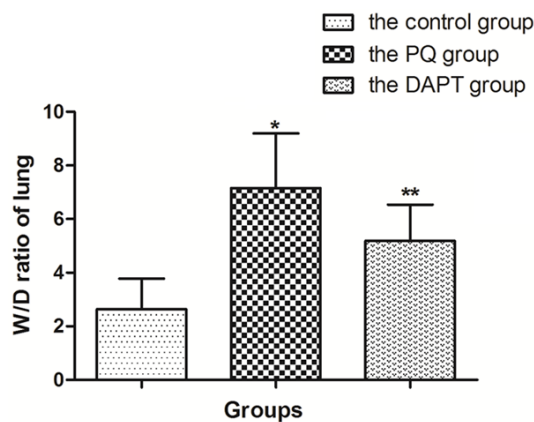


Figure 2. Paraquat increases the wet/dry W/D lung ratio, which is attenuated by DAPT. The wet/dry W/D lung ratio was determined at 70°C for 72 h following paraquat (PQ) injection. Data are presented as the mean \pm standard deviation (SD). Compared with the PQ group, there was a significant decrease in the lung W/D ratio in the DAPT treated group. $P < 0.05$ indicates a statistically significant difference, $n = 6$ per group.

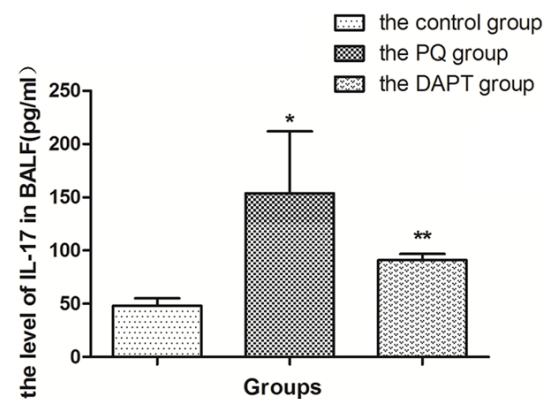


Figure 3. Paraquat increases the expression of interleukin 17A (IL-17A) in BALF, which is attenuated by DAPT. In the PQ-treated group, the levels of IL-17A in BALF were significantly increased compared with the control group ($P < 0.05$). However, relative to the PQ group, the DAPT group expressed obviously decreased levels of IL-17A. Data are presented as the mean \pm SD ($n = 6$ /group). $P < 0.05$ indicates a statistically significant difference.

the effects of DAPT, the expression of IL-17A was determined in bronchoalveolar lavage fluid (BALF) by ELISA. As shown in **Figure 3**, after PQ injection, the levels of IL-17A were significantly increased in the PQ-treated group compared with the control group. However, relative to the PQ group, the DAPT group expressed obviously decreased levels of IL-17A. These findings confirmed the hypothesis that DAPT regulated the expression of the inflammatory cytokines.

DAPT inhibits the percentage of Th17 cells in the spleen of PQ induced ALI

Flow cytometry was used to examine the percentage of Th17 Cells among total CD4^{+} T cells in the spleen. As shown in **Figure 4**, the PQ group exhibited an increase in Th17 Cells, compared with the control group. In contrast, the percentage of Th17 cells was significantly reduced in the DAPT group compared with those in the PQ group. These results indicate that

DAPT alleviates Th17 cells in PQ-induced ALI

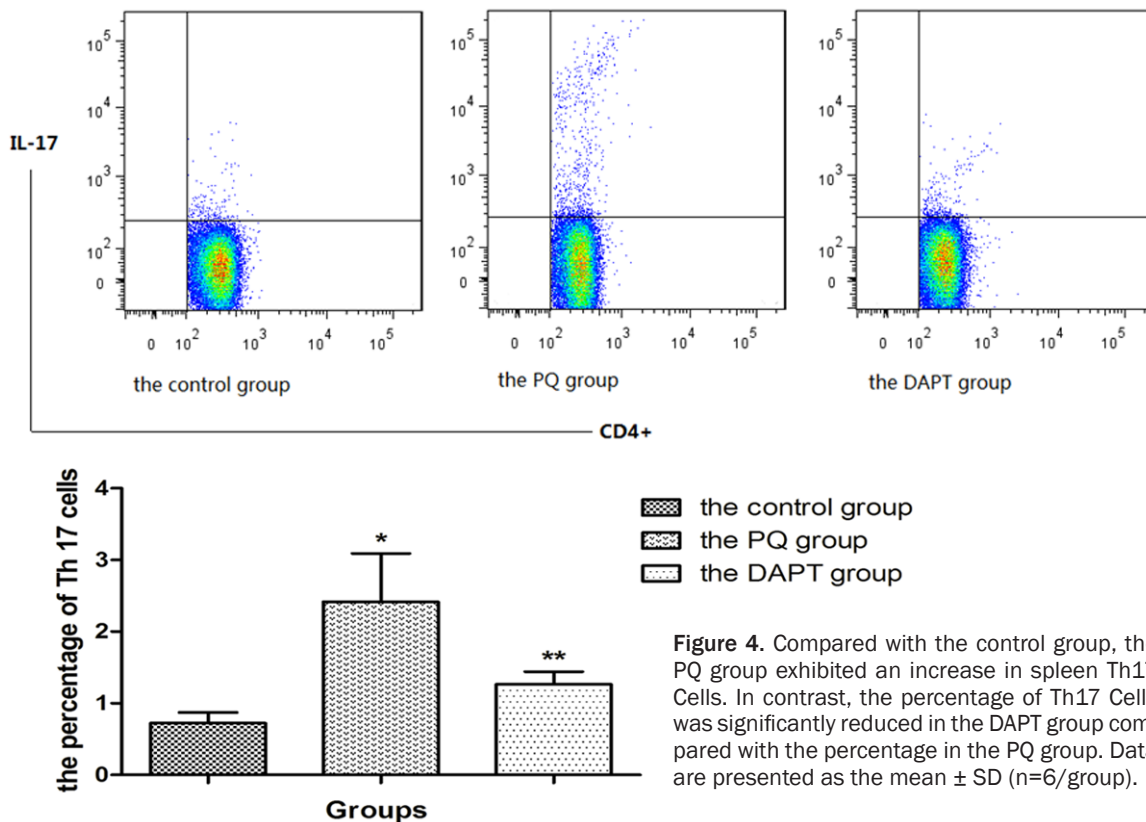


Figure 4. Compared with the control group, the PQ group exhibited an increase in spleen Th17 Cells. In contrast, the percentage of Th17 Cells was significantly reduced in the DAPT group compared with the percentage in the PQ group. Data are presented as the mean \pm SD (n=6/group).

DAPT could affect the percentage of Th17 cells in the spleen of PQ-treated animals.

DAPT effects the mRNA expression of ROR γ t in lung tissue in the PQ-induced mouse model of ALI

To evaluate the effects of DAPT on Th17 transcription factor retinoid-related orphan nuclear receptor (ROR γ t) in PQ-induced ALI, the mRNA expression of ROR γ t was analyzed by quantitative real-time PCR. As shown in **Figure 5**, compared with the control group, PQ-administrated mice in the PQ group revealed increased mRNA expression of ROR γ t. Conversely, DAPT treatment decreased the mRNA expression of ROR γ t compared to the PQ group.

Discussion

Due to the lack of an antidote or effective treatment, PQ poisoning is a life-threatening clinical syndrome characterized by acute respiratory failure with extremely high mortality [14-16]. Although there have been many animal and clinical studies on PQ-associated ALI, the underlying mechanism is still not conclusively

known [17, 18]. However, studies have demonstrated that the activation of multiple inflammatory cells plays an important role in the development and outcome of PQ-induced ALI [19]. As an inflammatory cytokine, accumulating evidence has suggested that IL-17 is associated with the pathogenesis of multiple inflammatory diseases, including PQ-induced ALI [20].

Th17 cells are regarded as having a role in various inflammatory diseases such as acute lung injury which are characterized by the secretion of IL-17 and their expression of ROR γ t [21]. In our study, we found that the concentration of IL-17A in BALF was significantly increased in mice with PQ-induced ALI compared with the control group. Meanwhile, Th17 cells played a critical role in the inflammation of PQ-induced ALI, which upregulated the proportion of Th17 cells in the CD4⁺ T cells of mice spleens in the PQ group compared with those from the control group.

Interestingly, Notch signaling can induce the differentiation of Th17 cells and promote the expansion of IL-17 in the pathogenesis of several inflammatory diseases. As one of the most

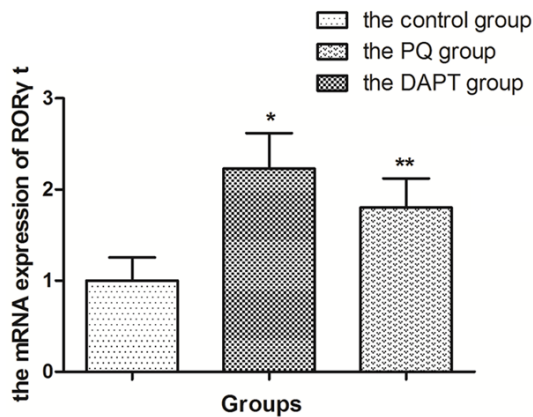


Figure 5. Compared with the control group, the mRNA levels of RORγt were significantly increased in the PQ-treated group. Conversely, DAPT treatment decreased the mRNA levels of RORγt compared with the PQ group. Data are presented as the mean ± SD (n=6/group).

widely studied intracellular signaling pathways, the Notch signaling pathway plays a key role in the pathogenesis of early lung injury, as shown by Zhu and colleagues, who demonstrated that the Notch signaling pathway is involved in angiogenesis in the process of smoke inhalation injury in a rat model [22]. Similarly, Han and colleagues have shown that the Notch signaling pathway is involved in zymosan-induced lung injury, and that the inhibition of this signaling pathway can ameliorate the expression of inflammatory cytokines and reduce local inflammatory lung damage [23]. These previous studies confirmed that a blocker of Notch signaling would inhibit the expression of Th17 cells.

Recently, increasing evidence has demonstrated that Notch signaling pathways play a critical role in the differentiation and activation of Th17 cells [24].

In the present study, our data suggest that the inhibition of Notch signaling by DAPT effectively blocks the differentiation of Th17 cells by regulating the level of IL-17 and the expression of RORγt. First, our study showed that DAPT could suppress the differentiation of Th17 cells in mice with PQ-induced ALI. By means of flow cytometry, we observed that the PQ group exhibited a significant increase in Th17 cells, and we further found that DAPT can affect the percentage of Th17 Cells in the spleen of treated

mice. Meanwhile, further investigation demonstrated that DAPT could also regulate the level of IL-17A in BALF. As important as the inflammatory cytokines are, accumulating evidence has demonstrated that IL-17A plays a crucial role in the pathogenesis of inflammatory disease [25]. In our study, we observed that the expression of the proinflammatory cytokines IL-17A in BALF was significantly increased in mice with PQ-induced ALI. However, there was a significant decrease in the DAPT group. The above results reveal the interesting phenomenon that DAPT can regulate the level of IL-17A in mice with PQ-induced ALI. Furthermore, as a unique transcription factor, Th17 cells express RORγt [26]. Our study demonstrated that the γ-secretase inhibitor (DAPT) could suppress the expression of RORγt mRNA in mice with PQ-administrated ALI, which suggests that Notch signaling can regulate the differentiation of Th17 cells via RORγt. In addition, we also found that the inhibition of notch signaling can alleviate lung inflammation and tissue damage. Lung histopathology and the W/D ratio in DAPT group were markedly alleviated. These results are in accordance with previous studies [27].

Taken together, in a mouse model of paraquat (PQ)-induced acute lung injury (ALI), the Notch signaling pathway was shown to be involved in lung inflammation, and its role is related to the secretion of inflammatory cytokines. γ-secretase inhibitor (DAPT) can block the Notch signaling and suppress the level of IL-17A, which provides a new avenue for the prevention and treatment of PQ-induced ALI. Nevertheless, there are of course some limitations to our present study. Further studies are needed to elucidate the possible mechanisms of the process described here.

Acknowledgements

This study was supported by grants from the Open Issues of The Guangxi Talent Highland for Emergency and Rescue Medicine, Guangxi Colleges and Universities, Key Laboratory of Emergency Medicine Research (Ref: GXJZ201-503 and GXJZ201426), and the National Natural Science Foundation of China (No: 81560-309). We thank Simon Teteris, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English test of a draft of this manuscript.

Disclosure of conflict of interest

None.

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References

- [1] Zhang Q, Wei-Zhen WU, Yuan-Qiang LU, Wang JZ, Shang AD, Yao F and Chen Y. Successful treatment of patients with paraquat intoxication: three case reports and review of the literature. *J Zhejiang Univ Sci B* 2012; 13: 413-418.
- [2] Kim KS, Suh GJ, Kwon WY, Kwak YH, Lee K, Lee HJ, Jeong KY and Lee MW. Antioxidant effects of selenium on lung injury in paraquat intoxicated rats. *Clin Toxicol* 2012; 50: 749-753.
- [3] Dinis-Oliveira RJ, Duarte JA, Sánchez-Navarro A, Remião F, Bastos ML and Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. *Crit Rev Toxicol* 2008; 38: 13-71.
- [4] Ming R, Wang YM, Jing Z, Zhao J, Zhao ZM, Zhang TF, He J, Ren SP and Peng SQ. Metallothioneins attenuate paraquat-induced acute lung injury in mice through the mechanisms of anti-oxidation and anti-apoptosis. *Food Chem Toxicol* 2014; 73: 140-147.
- [5] Jiang Z, Zhou Q, Gu C, Li D and Zhu L. Depletion of circulating monocytes suppresses IL-17 and HMGB1 expression in mice with LPS-induced acute lung injury. *Am J Physiol Lung Cell Mol Physiol* 2016; 312: L231-L242.
- [6] Zhang F, Li MY, Lan YT and Wang CB. Imbalance of Th17/Tregs in rats with smoke inhalation-induced acute lung injury. *Sci Rep* 2016; 6: 21348.
- [7] Zhu Y, Wang W and Wang X. Roles of transcriptional factor 7 in production of inflammatory factors for lung diseases. *J Transl Med* 2015; 13: 273.
- [8] Kleinjan A, Bruijn MD, Nimwegen MV, Bergen I, Hoogsteden HC, Amsen D and Hendriks RW. The Notch mediator RBP-J in CD4 T cells plays a crucial role in the induction of allergic asthma in mice. *J Allergy Clin Immunol* 2013; 131: AB147-AB147.
- [9] Radtke F, Macdonald HR and Tacchinicottier F. Regulation of innate and adaptive immunity by Notch. *Nat Rev Immunol* 2013; 13: 427-437.
- [10] Weng C, Lei C, Jia X, Zheng R, Yue H, Zhu T, Li C and Zhang W. Anti-DII4 antibody inhibits the differentiation of Th17 cells in asthmatic mice. *Inflammation* 2017; 40: 1-8.
- [11] Jiao Z, Wang W, Hua S, Liu M, Wang H, Wang X, Chen Y, Xu H and Lu L. Blockade of Notch signaling ameliorates murine collagen-induced arthritis via suppressing Th1 and Th17 cell responses. *Am J Pathol* 2014; 184: 1085-1093.
- [12] Du J, Li X, Lin C and He X. Protective Effects of arachidonic acid against paraquat-induced pulmonary injury. *Inflammation* 2015; 38: 1458-1463.
- [13] Zhang J, Li C and Guo S. Effects of inhaled inactivated Mycobacterium phlei on airway inflammation in mouse asthmatic models. *J Aerosol Med Pulm Drug Deliv* 2012; 25: 96.
- [14] Moon JM, Chun BJ and Cho YS. The characteristics of emergency department presentations related to acute herbicide or insecticide poisoning in South Korea between 2011 and 2014. *J Toxicol Environ Health A* 2016; 79: 466-76.
- [15] ZE S. Role of antioxidants in paraquat toxicity. *Toxicology* 2002; 180: 65-77.
- [16] Kang C, Kim SC, Lee SH, Jeong JH, Kim DS and Kim DH. Absolute lymphocyte count as a predictor of mortality in emergency department patients with paraquat poisoning. *PLoS One* 2013; 8: e78160.
- [17] Huang Y, Yin W, Yu H, Wang Y, Liu C, Xiong J and Hao L. Combined treatment with bone marrow mesenchymal stem cells and methylprednisolone in paraquat-induced acute lung injury. *BMC Emerg Med* 2013; 13 Suppl 1: S5.
- [18] Liu W, Shan LP, Dong XS and Liu Z. Toll-like receptor 4 implicated in acute lung injury induced by paraquat poisoning in mice. *Int J Clin Exp Med* 2014; 7: 3392-3397.
- [19] Hu X, Shen H, Wang Y and Zhao M. Liver X receptor agonist T0901317 attenuates paraquat-induced acute lung injury through inhibition of NF- κ B and JNK/p38 MAPK signal pathways. *Biomed Res Int* 2017; 2017: 4652695.
- [20] Song HW, Yang C, Liu W, Liu XW, Liu Z and Gao F. Interleukin-17A plays the same role on mice acute lung injury respectively induced by lipopolysaccharide and paraquat. *Inflammation* 2017; 40: 1509-1519.
- [21] Zepp J, Wu L, Li X. IL-17 receptor signaling and T helper 17-mediated autoimmune demyelinating disease. *Trends Immunol* 2011; 32: 232-9.
- [22] Zhu F, Wang J, Qiu X, Li J and Xia Z. Smoke inhalation injury repaired by a bone marrow-derived mesenchymal stem cell paracrine mechanism: angiogenesis involving the Notch signaling pathway. *J Trauma Acute Care Surg* 2015; 78: 565-72.
- [23] Han H, Gong G, Bai X, Lin YC, Sun J, Wang W, Zhao Y, Yang L, Wang X and Zhang Z. Inhibition

- of notch signaling protects mouse lung against zymosan-induced injury. *Shock* 2013; 40: 312-9.
- [24] Keerthivasan S, Suleiman R, Lawlor R, Roderick J, Bates T, Minter L, Anguita J, Juncadella I, Nickoloff BJ, Le Poole IC, Miele L, Osborne BA. Notch signaling regulates mouse and human Th17 differentiation. *J Immunol* 2011; 187: 692-701.
- [25] Shao L, Cong Z, Li X, Zou H, Cao L and Guo Y. Changes in levels of IL-9, IL-17, IFN- γ , dendritic cell numbers and TLR expression in peripheral blood in asthmatic children with *Mycoplasma pneumoniae* infection. *Int J Clin Exp Pathol* 2015; 8: 5263-5272.
- [26] Toussiot E. The IL23/Th17 pathway as a therapeutic target in chronic inflammatory diseases. *Inflamm Allergy Drug Targets* 2012; 11: 159-68.
- [27] Han H, Gong G, Bai X, Lin YC, Sun J, Wang W, Zhao Y, Yang L, Wang X and Zhang Z. Inhibition of notch signaling protects mouse lung against zymosan-induced injury. *Shock* 2013; 40: 312-319.