5-Aminosalicylic acid Attenuates Paraquat-induced Lung Fibroblasts Activation and Pulmonary Fibrosis of Rats

Hui Chen

Hebei Medical University No2 Hospital: Second Hospital of Hebei Medical University

Jinfeng Cui

The second hospital of Hebei medical university

Juan Wang

The second hospital of Hebei medical university

Yuan Wang

The second hospital of Hebei medical university

Fei Tong

Second Hospital of Hebei Medical University

Yingping Tian

Second Hospital of Hebei Medical University

Yu Gong

Second hospital of Hebei medical university

Yu Ma

Second hospital of Hebei medical university

Liang Liu

Second hospital of Hebei medical university

Xianghong Zhang (✉ zhangxianghong2008@163.com)

Hebei medical university

Research Article

Keywords: 5-ASA, Paraquat, PPAR γ , pulmonary fibrosis, TGF- β /Smad3

Posted Date: February 19th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-238806/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Molecular Medicine Reports on December 15th, 2021. See the published version at <https://doi.org/10.3892/mmr.2021.12574>.

Abstract

Pulmonary fibrosis is one of the most common complications of paraquat (PQ) poisoning, which becomes the focus of treatment. More and more studies have found that 5-Aminosalicylic acid (5-ASA) may be a prospective therapy against fibrotic diseases. In the present study, we observed whether 5-ASA could attenuate the pulmonary fibrosis in PQ-treated rats and human lung fibroblasts (WI38VA13) cells, and subsequently explored the possible underlying mechanisms. Wistar rats were divided into control group, 5-ASA group, PQ group and PQ + 5-ASA group. Rats were sacrificed on 3, 7, 14, and 28 days after PQ treatment. We observed pulmonary histopathological changes and fibrosis formation among different groups through hematoxylin and eosin (H&E) and Masson staining and TGF- β 1, p-Smad3 and the peroxisome proliferator activated receptor γ (PPAR γ) pulmonary content via immunohistochemical staining and Western blot. In addition, human lung fibroblasts WI38VA13 were also divided into control group, PQ group, 5-ASA group and PQ + 5-ASA group. And the role of TGF- β 1 signaling pathway regulated factors (TGF- β 1, p-Smad3 and PPAR γ) were explored. Treatment with 5-ASA significantly inhibited the PQ-induced activation of TGF- β 1 signaling pathway in human lung fibroblasts WI38VA13 cells. In conclusion, the results of this study suggested that 5-ASA has potential value in the treatment of PQ-induced pulmonary fibrosis via suppressing the activation of TGF- β 1 signaling pathway.

Background

As an effective herbicide, Paraquat (PQ, 1,1'-dimethyl-4,4'-bipyridinium) is a highly toxic pro-oxidant that is widely used throughout the world. Up to now, human PQ intoxication by accidentally exposure or with suicide intention could still be seen. PQ poisoning induces multi-organ failure involving lung, gastrointestinal tract, pancreas, kidney, liver, heart, and brain injury. Pulmonary fibrosis is the most typical feature of PQ poisoning and continues from several days to weeks after PQ ingestion.(Shadnia et al., 2018)

Transforming growth factor- β 1 (TGF- β 1) has been reported to be the key growth factor that initiates tissue repair and its sustained production is involved in the development of tissue fibrosis.(Han et al., 2015) TGF- β 1 could activate down-stream transcription factor Smad, and then trigger the intracellular signaling pathway.(Hu et al., 2018) Early Study by Sato et al showed that targeted disruption of TGF- β 1/Smad3 signaling protected against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction in mice lacking Smad3 (Smad3 ex8/ex8).(Sato et al., 2003) There have been several other studies with different models of kidney disease further confirmed the central role of Smad3 pathway in the pathogenesis of interstitial fibrosis.(Loboda et al., 2016, Sisto et al., 2018) The peroxisome proliferator activated receptor γ (PPAR γ) is well known for its ability to regulate glucose and lipid metabolism. It has been recently found that the interaction of TGF β 1 and PPAR γ is involved in the development of fibrosis. TGF β 1 controlled PPAR γ expression, transcriptional potential, and activity partly through Smad3 signaling in murine lung fibroblasts.(Ramirez et al., 2012) PPAR γ is an inhibitory regulator of TGF β 1-Smad pathway, while TGF β 1 induces fibrosis-related genes suppressing PPAR γ . PPAR γ is an important regulator of TGF β 1-associated diseases.(Calvier et al., 2017)

5-Aminosalicylic acid (5-ASA) is an anti-inflammatory agent. Over the past decades, 5-ASA preparations have been specific and first-line therapeutic drugs for mild to moderate active inflammatory bowel disease (IBD). 5-ASA was found to reduce TGF- β signaling as judged by the reduction in TGF- β specific reporter gene activity.(Rousseaux et al., 2013) The intestinal anti-inflammatory effect of 5-ASA was dependent on PPAR γ (Rousseaux et al., 2005, Sarraf et al., 1998) and its anti-neoplastic effect in the intestine was also mediated by PPAR γ . Thus, it is clear that PPAR γ is the key mediator for anti-inflammatory and antineoplastic effects of 5-ASA. As PPAR γ is also involved in the development of fibrotic diseases, it is interesting to see if 5-ASA could take a part in the interaction of PPAR γ and TGF- β 1/ Smad3 pathway in the pathogenesis pulmonary fibrosis and to be used as a potential drug for PQ poisoning.

Therefore, in the present study, we firstly investigate whether 5-ASA exerts protective effect against PQ-induced pulmonary fibrosis. Secondly, we explore the putative mechanism of 5-ASA in preventing PQ-induced pulmonary fibrosis. This study provides a new clue for the treatment of PQ poisoning.

Materials And Methods

Reagents

We bought paraquat (33.5%) from Syngenta Crop Protection (Nantong, Jiangsu, China). 5-ASA (98.5%, chemical purity) was purchased from J&K Science and Technology Co., Ltd. The main antibodies used were as follows: rabbit anti-human PPAR γ , rabbit anti-human TGF- β 1 antibodies (Bioworld, Bloomington, USA) and rabbit anti-human phospho-Smad3 antibody (Affinity, Cincinnati, OH, USA).

Animals

In this study, 100 healthy male Wistar rats aged 6-8 weeks and weighing 180-220 g were reared at Animal Center of Hebei Medical University. The animals were placed in a ventilated room of 22 ± 2 °C for 12 hr of light/dark cycle. Feed and water the rats at will. All animal experiments conform to the guidelines of the Ethics Committee for Laboratory Animals of Hebei Medical University.

Animal models and tissue sampling

The Wistar rats were divided randomly to four groups: control group, PQ group, 5-ASA group and PQ+5-ASA group with twenty-five rats in each group. The concentration of PQ in this study was selected based on the results of our pre-experiments and the related literatures.(Zheng et al., 2017, Tang et al., 2017) On the first day, the rats in the PQ and PQ+5-ASA groups were given doses of 80 mg/kg PQ by gavage, whereas in control and 5-ASA groups, the rats were treated with distilled water, 2 hr later, equal amounts of distilled water were given for control and PQ groups, while 30 mg/kg 5-ASA in distilled water were intragastrically administered respectively for 5-ASA and PQ+5-ASA group. On the second day, only equal amounts of distilled water were given for control and PQ groups, at the same time, 30 mg/kg 5-ASA were given for 5-ASA and PQ+5-ASA groups respectively, the steps of the second day repeats once a day for up

to 7 days. The rats were then killed for preparation of cervical dislocation specimens after anesthesia at 3, 7, 14 and 28 days after PQ administration. The general pathological changes of rats in each group were observed, including size, congestion and dot bleeding. To distinguish the rats' left and right lungs, each left lung was placed in a frozen pipette and stored in a -70°C liquid nitrogen freezer to detect the Hydroxyproline (HYP). The right lung was immersed in 10% formalin and then embedded in paraffin.

Lung coefficient

We took the whole lung and the whole trachea of the rats, cut the trachea between the 5 and 6 cartilage rings above the tracheal bifurcation, used the clean filter paper to absorb the blood and tissue fluid on the lung surface, and then weighed that with high precision balance. Pulmonary coefficient calculation method is as follows: lung coefficient (LI) = total lung wet weight (mg) / body weight (g)×100%.

Histopathologic examinations

The right lung tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned (thickness of 5µm), and slides were stained with hematoxylin and eosin (H&E) and then examined under light microscope (Leica Microsystems Nussloch GmbH, RM2245, Nussloch, Germany) by an experienced pathologist who was blinded to the treatment each animal had received.

Masson's trichrome stain

The slides were deparaffinized and subjected to Masson staining for fibrosis detection. The slides with the Masson trichrome stain were observed using a Leica microscope (Leica Microsystems Nussloch GmbH, RM2245, Nussloch, Germany), the smooth muscle cell cytoplasm was stained red, while the collagenous fibrous tissue was stained blue.

Hydroxyproline (HYP) determination

According to the manufacturer's instructions for the hydroxyproline assay kit (Solarbio, Beijing, China), lung tissues were isolated to determine the optical density (OD value) of the samples at a wavelength of 550 nm in a microplate reader, and the level of hydroxyproline was calculated accordingly.

Cell culture and treatment

Human lung fibroblasts WI38VA13 purchased from ATCC were grown in DMEM/F12 at 37°C supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and streptomycin in 5% CO₂/95% air. When cells reached 80% confluence, they were randomly divided into four groups: Control group, PQ group, 5-ASA group, and PQ+5-ASA group. The cells were incubated with 200µM PQ for 12, 24 and 48 hr, with and without pretreatment with 5-ASA (10mM) for 2 hr. Cells were collected for 12, 24, and 48 hr after PQ treatment.

Western blotting analyses

After treatment, cells were washed with ice-cold PBS. Total cell proteins were then extracted using lysis buffer (1% Triton X-100, 150 mM NaCl, 2 mM EDTA, 50 mM Tris-HCl, 10% phosphatase inhibitors and 1% cocktail). 40 µg of protein was used for SDS-PAGE, transferred to PVDF membrane. Subsequently, the membranes were blocked with 5% skim milk and incubated with specific antibodies at 1:1000 dilution at 4°C. After washing, the membranes were incubated with secondary antibody at 1:5000 dilution and visualized by ECL chemiluminescent detection system. Bands density was quantified using Snygene-Image System and normalized to β-Actin.

Immunohistochemical Staining

Sections (4µm thick) were prepared from paraffin blocks. After deparaffinization, antigen retrieval was performed under citrate buffer for 15 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 10 min. Incubation with primary antibodies against TGF-β (1:100) and PPARγ (1:100) was conducted overnight at 4°C in a humidified chamber. After the slices were washed in 0.01mol/L phosphate-buffered saline, the operation followed was done as described previously with PowerVision-9000 kit (GBI, Washington, USA) for immunohistological staining.(Wei et al., 2010) The primary antibody was replaced by phosphate-buffered saline as a negative control. All slides were scored by an experienced pathologist.

Statistical analysis

Statistical analysis was performed by the one way analysis of variance (ANOVA) with SPSS 16.0 software. The results were presented as means ± standard deviation. Values were considered statistically significant when $P < 0.05$.

Results

The effect of general condition after PQ exposure

General poisoning manifestations including sluggish, lethargy, irritability and bloody discharge in mouth, nose and eyes could be found in rats 2 hours after PQ exposure. The body weights of the rats after PQ exposure were all significantly decreased with decrease in water and food intake. Systemically, dyspnea, abdominal breathing, perioral cyanosis in respiratory system, diarrhea in alimentary system as well as oliguria, anuresis, hematuria in urinary system could also be seen in PQ exposure rats. The poisoning manifestations, both general and systemic, were alleviated in PQ+5-ASA groups.

Relative body weight gradually increases with time in 5-ASA group. It has no change compared with the control group($P > 0.05$). After the PQ exposure, the relative weight decreased to minimum at 3d ($P < 0.05$), then gradually increased to the control level at 14d ($P > 0.05$). In PQ+5-ASA group, relative body weight decreased to a minimum at 3d. It had statistical significance when compared with control group and 5-ASA group at 3d ($P < 0.05$, Fig.1). So the PQ-induced relative body weight decrease could be improved by 5-ASA.

5-ASA attenuates PQ-induced pulmonary damages

Grossly, lungs in control and 5-ASA groups were all normal in size and pink in color, while lungs in PQ group were significantly increased in size, congestion and dot bleeding could be seen within 7 days of PQ exposure and became grayish with uneven surface 28 days after PQ exposure. The gross changes of the lungs in PQ+5-ASA groups were significantly alleviated and no obvious bleeding could be seen and lung surface were smooth. Lung coefficient measurement further confirmed 5-ASA treatment could alleviate the injurious changes of PQ in lungs (Fig. 2).

Histologically, the morphological changes of lungs of rats in different groups were evaluated with H&E staining (Fig. 3). Except occasionally a few phagocytes in the lumens of alveoli, no injurious changes including edema, congestion, bleeding and inflammatory changes could be seen in the lungs in control and 5-ASA groups. Alveolitis were observed in lungs of the rats after PQ exposure. Congestion, edema and inflammatory cell infiltration could be seen in bronchial and alveolar walls at 3 and 7 days after PQ exposure. Alveolar septa thickening with alveolar lumen narrowing, diffuse pulmonary hemorrhage, hyaline membrane formation could be seen at 14 day after PQ exposure. At the later stage, we also observed fibroblast proliferation, increase of collagen fibers and fibrous thickening of the alveolar walls at 28 day after PQ exposure. All of those pulmonary injury changes seen in the lungs of rats in PQ group were significantly attenuated in the two 5-ASA treatment group, evidenced by decreased in the degree of congestion, inflammatory cell infiltration, bleeding at early stage(<14 days) and fibrous proliferation at late stage (28 days) after PQ exposure.

5-ASA attenuates PQ-induced fibrosis in lung tissues of rats

Pulmonary fibrosis is characterized by the accumulation of collagens. HPY is a non-essential amino acid found in collagen, playing a crucial role in collagen synthesis, and is frequently used as a biomarker of tissue fibrosis. We measured HPY contents changes of the lung tissue in different groups. The results showed that HYP content was significantly increased in lung tissues of the rats for 7 days after PQ treatment, and reached to a peak at the 28 day. Compared to the HYP changes in PQ group, the emerging time of the increase of HYP content was delayed by 7 days after PQ+5-ASA treatment and HYP contents of lung tissue were significantly lower in the lung tissues of rats at the 14 and 28 days after PQ+5-ASA treatment (Fig. 4), suggesting that 5-ASA treatment could significantly alleviate the degree of collagen accumulation induced by PQ.

With Masson trichrome staining, the collagen fibers were dyed blue. The results (Fig. 5) showed that as compared with that in control group (Fig. 5A) and 5-ASA group (Fig. 5B), the amount of blue stained collagen fibers in the lung tissues of rats in PQ group (Fig. 5C) was increased. The collagen fibers were mainly concentrated in the alveolar thickening area and bronchioles. With 5-ASA treatment, the amount of collagen fibers of lung tissue in PQ+5-ASA groups were all less than those in PQ alone (Fig. 5D), further confirmed the alleviating effect of 5-ASA on the increase of collagen fiber amount induced by PQ.

Taken together, these data showed that 5-ASA treatment could attenuate the collagen accumulation of lung tissue induced by PQ. It suggested that 5-ASA has a protective effect in PQ-induced pulmonary fibrosis of rats.

5-ASA attenuate the upregulation of TGF- β 1, p-Smad3, and the reduction of PPAR γ induced by PQ in lung tissue of rats in vivo and human lung fibroblasts WI38VA13 cells in vitro

We further investigated the expression pattern of fibrosis related factors in lung tissue of rats by immunohistochemical staining to evaluate the possible anti-fibrotic mechanism of 5-ASA. The immunohistochemical staining results showed that TGF- β 1 expression was increased from 3 to 28 days after PQ exposure. Compared to that of PQ group, the expression level of TGF- β 1 was decreased in PQ+5-ASA groups at every measured time point (Fig. 6). The expression of PPAR γ in the lung tissue of rats in 5-ASA group was a little bit increased while in PQ group was obviously decreased than that in control group. And this downregulation of PPAR γ induced by PQ could be partly prevented by 5-ASA treatment in lung tissue of rats (Fig. 7). In addition, Western blotting results showed that PQ exposure significantly increased the phosphorylation level of Smad3 of lung tissues compared with that in control group at all the time points ($P < 0.05$). The p-Smad3 level was significantly down-regulated in PQ+5-ASA-treated rats lung tissue compared with the PQ treatment group (Fig. 8). Thus, taking all these together, the results suggest that the inhibition effect of 5-ASA on pulmonary fibrosis induced by PQ be achieved via attenuating the upregulation of TGF- β 1, p-Smad3 and downregulation of PPAR γ .

Studies have shown that myofibroblast proliferation of lung tissue of patients is the bases of pulmonary fibrosis.(Kuhn & McDonald, 1991, Pache et al., 1998) Thus, based on the aforementioned results, we further explored the effect of 5-ASA on the TGF- β 1 pathway after PQ exposure in human lung fibroblasts WI38VA13 cells *in vitro*. The results as determined by Western blot were consistent with that *in vivo* and revealed that pretreatment with 5-ASA significantly restore the reduction of PPAR γ (Fig. 9A) and reverse the increase of TGF- β 1(Fig. 9B) and p-Smad3 (Fig. 9C) induced by PQ.

Collectively, these data demonstrated that 5-ASA treatment could attenuate PQ-induced pulmonary fibrosis progression through up-regulating the expression of PPAR γ and inhibiting the activation of TGF- β 1/Smad3 signaling pathway.

Discussion

Pulmonary fibrosis is an irreversible stage of pathologic development of PQ poisoning, leading to high mortality.(Dong et al., 2016) So far, no safe and effective treatments were found to reverse fibrosis process. In this study, we found that intragastric administration of PQ induced significant injury and fibrotic changes in the lungs of experimental rats including congestion, edema, hemorrhage, increased lung coefficient, the increased HYP content, accumulation of collagen, increase of collagen fibers and fibrous thickening of the alveolar walls *etc*. 5-ASA treatment could significantly alleviated the pulmonary injury and fibrotic changes induced by PQ in rats, evidenced by decreased in the degree of congestion, inflammatory cell infiltration, hemorrhage, lung coefficient increase, collagen accumulation and collagen

fiber increase. Thus, our study confirmed that 5-ASA could significantly attenuated the injury and fibrotic changes induced by PQ and suggesting 5-ASA might be used in the alleviation of pulmonary fibrosis in PQ poisoning.

TGF- β 1 plays a crucial role in the induction of fibrosis. The TGF- β 1 signal exerts its biological effects via the TGF- β 1/SMAD/Snail signaling pathway, playing an important pathogenic role in several fibrotic diseases.(Sisto et al., 2018, Zhou et al., 2017) In this study with rats, we also confirmed the upregulation of TGF- β 1 and Smad3 activation evidence by increased pSmad3 level after PQ exposure, suggesting that TGF- β 1/Smad signaling pathway be involved in the fibrotic changes induced by PQ. The results were in accordance with that in the literature on tissue fibrosis.(Xue et al., 2011)

PPARs are recognized as versatile members of the ligand-activated nuclear hormone receptor superfamily of transcription factors that includes receptors for steroids, thyroid hormone, retinoic acid, and vitamin D among others.(Rabinovitch, 2010) There are three subtypes of PPARs: PPAR α , PPAR β/δ , and PPAR γ . Of the three PPARs identified to date, PPAR γ represents the most promising PPAR target in lung diseases in view of emerging reports implicating this molecule in various pulmonary processes.(Standiford et al., 2005) More recently, it has been reported that PPAR γ activators could inhibit TGF- β 1-induced myofibroblast transdifferentiation.(Kulkarni et al., 2011) In addition, in diseased tissues, PPAR γ expression has been shown to relate inversely with that of TGF- β 1.(Tang et al., 2017) Thus, it appears that the balance between TGF- β 1 and PPAR γ may determine whether fibrogenesis predominates after tissue injury. In this study, we found that the expression of PPAR γ was significantly decreased in lung tissues of rats after PQ intragastric treatment. At the same time, we also found that PQ exposure decreased the expression of PPAR γ in human lung fibroblasts WI38VA13 cells *in vitro*. Therefore, our studies suggested that PPAR γ involved in the regulation of TGF- β 1/Smad3 signaling pathway in PQ-induced pulmonary fibrosis.

5-ASA is an anti-inflammatory agent commonly used for the treatment of inflammatory bowel disease. (Moran et al., 2018) Although the exact mechanisms of action of 5-ASA are not completely elucidated, recent studies have revealed that the basic mechanism of action of 5-ASA was relying on increased expression of PPAR γ .(Serra et al., 2016, Rousseaux et al., 2005) As a ligand of PPAR, 5-ASA could increase PPAR γ expression, promote its translocation from the cytoplasm to the nucleus, and induce activation of down-stream signaling pathway.(Dubuquoy et al., 2006, Rousseaux et al., 2013)

Our results had showed that 5-ASA treatment could significantly relieve the PQ-induced pulmonary fibrotic changes of rats. To explore the putative mechanism of the attenuating effects of 5-ASA on PQ induced pulmonary fibrotic changes, the expression and interaction of TGF- β 1/Smad3 signaling and PPAR γ were studied both in *vivo* with rats and in *vitro* with human lung fibroblasts WI38VA13 cells. The results showed that 5-ASA treatment could dramatically prevent the up-regulation of TGF- β 1, the phosphorylation level of smad3 and the down-regulation of PPAR γ induced by PQ in human lung fibroblasts WI38VA13 cells. Collectively, these data directly suggest that 5-ASA treatment could attenuate

PQ-induced pulmonary fibrosis progression through up-regulating the expression of PPAR γ and inhibiting the TGF- β 1/Smad3 signaling pathway.

In conclusion, our study showed for the first time that 5-ASA has significant inhibitory effects on pulmonary fibrosis progression in the PQ intoxication rats model, and these effects may be partly ascribed to the inhibition of TGF- β 1/Smad3 signaling pathway. Thus, 5-ASA has potential value in the treatment of PQ-induced pulmonary fibrosis.

Declarations

Acknowledgements

Not applicable.

Authors' contributions

Hui Chen and Jinfeng Cui conducted the experiments and wrote the manuscript, Juan Wang, Yuan Wang, Fei Tong, Yingping Tian, Yu Gong, Yu Ma and Liang Liu collected the data and performed the experiments, and Xianghong Zhang designed the study. The final version of the manuscript has been read and approved by all authors, and each author believes that the manuscript represents honest work.

Compliance with Ethical Standards

Conflict of interest

All authors declare that there is no conflict of interest.

Ethical Approval

All of the experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH Publication No.8023, Revised 1978) and were reviewed and approved by the Ethics Committee for the Use of Experimental Animals at Hebei Medical University (Permit number: 1608303)

References

- Calvier, L, Chouvarine, P, Legchenko, E, et al. (2017) PPAR γ Links BMP2 and TGF β 1 Pathways in Vascular Smooth Muscle Cells, Regulating Cell Proliferation and Glucose Metabolism. *Cell Metab* 25: 1118-1134 e1117
- Dong, J, Yu, X, Porter, DW, Battelli, LA, Kashon, ML and Ma, Q (2016) Common and distinct mechanisms of induced pulmonary fibrosis by particulate and soluble chemical fibrogenic agents. *Arch Toxicol* 90: 385-402

- Dubuquoy, L, Rousseaux, C, Thuru, X, et al. (2006) PPARgamma as a new therapeutic target in inflammatory bowel diseases. *Gut* 55: 1341-1349
- Han, YY, Shen, P and Chang, WX (2015) Involvement of epithelial-to-mesenchymal transition and associated transforming growth factor-beta/Smad signaling in paraquat-induced pulmonary fibrosis. *Mol Med Rep* 12: 7979-7984
- Hu, Z, Qin, F, Gao, S, Zhen, Y, Huang, D and Dong, L (2018) Paeoniflorin exerts protective effect on radiation-induced hepatic fibrosis in rats via TGF-beta1/Smads signaling pathway. *Am J Transl Res* 10: 1012-1021
- Kuhn, C and McDonald, JA (1991) The roles of the myofibroblast in idiopathic pulmonary fibrosis. Ultrastructural and immunohistochemical features of sites of active extracellular matrix synthesis. *Am J Pathol* 138: 1257-1265
- Kulkarni, AA, Thatcher, TH, Olsen, KC, Maggirwar, SB, Phipps, RP and Sime, PJ (2011) PPAR-gamma ligands repress TGFbeta-induced myofibroblast differentiation by targeting the PI3K/Akt pathway: implications for therapy of fibrosis. *PLoS One* 6: e15909
- Loboda, A, Sobczak, M, Jozkowicz, A and Dulak, J (2016) TGF-beta1/Smads and miR-21 in Renal Fibrosis and Inflammation. *Mediators Inflamm* 2016: 8319283
- Moran, CJ, Huang, H, Rivas, M, Kaplan, JL, Daly, MJ and Winter, HS (2018) Genetic variants in cellular transport do not affect mesalamine response in ulcerative colitis. *PLoS One* 13: e0192806
- Pache, JC, Christakos, PG, Gannon, DE, Mitchell, JJ, Low, RB and Leslie, KO (1998) Myofibroblasts in diffuse alveolar damage of the lung. *Mod Pathol* 11: 1064-1070
- Rabinovitch, M (2010) PPARgamma and the pathobiology of pulmonary arterial hypertension. *Adv Exp Med Biol* 661: 447-458
- Ramirez, A, Ballard, EN and Roman, J (2012) TGFbeta1 Controls PPARgamma Expression, Transcriptional Potential, and Activity, in Part, through Smad3 Signaling in Murine Lung Fibroblasts. *PPAR Res* 2012: 375876
- Rousseaux, C, El-Jamal, N, Fumery, M, et al. (2013) The 5-aminosalicylic acid antineoplastic effect in the intestine is mediated by PPARgamma. *Carcinogenesis* 34: 2580-2586
- Rousseaux, C, Lefebvre, B, Dubuquoy, L, et al. (2005) Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. *J Exp Med* 201: 1205-1215
- Sarraf, P, Mueller, E, Jones, D, et al. (1998) Differentiation and reversal of malignant changes in colon cancer through PPARgamma. *Nat Med* 4: 1046-1052

- Sato, M, Muragaki, Y, Saika, S, Roberts, AB and Ooshima, A (2003) Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. *J Clin Invest* 112: 1486-1494
- Serra, D, Almeida, LM and Dinis, TC (2016) Anti-inflammatory protection afforded by cyanidin-3-glucoside and resveratrol in human intestinal cells via Nrf2 and PPAR-gamma: Comparison with 5-aminosalicylic acid. *Chem Biol Interact* 260: 102-109
- Shadnia, S, Ebadollahi-Natanzi, A, Ahmadzadeh, S, Karami-Mohajeri, S, Pourshojaei, Y and Rahimi, HR (2018) Delayed death following paraquat poisoning: three case reports and a literature review. *Toxicol Res (Camb)* 7: 745-753
- Sisto, M, Lorusso, L, Ingravallo, G, Tamma, R, Ribatti, D and Lisi, S (2018) The TGF-beta1 Signaling Pathway as an Attractive Target in the Fibrosis Pathogenesis of Sjogren's Syndrome. *Mediators Inflamm* 2018: 1965935
- Standiford, TJ, Keshamouni, VG and Reddy, RC (2005) Peroxisome proliferator-activated receptor-gamma as a regulator of lung inflammation and repair. *Proc Am Thorac Soc* 2: 226-231
- Tang, H, Xiang, D, Wang, F, Mao, J, Tan, X and Wang, Y (2017) 5-ASA-loaded SiO₂ nanoparticles-a novel drug delivery system targeting therapy on ulcerative colitis in mice. *Mol Med Rep* 15: 1117-1122
- Wei, J, Ghosh, AK, Sargent, JL, et al. (2010) PPARgamma downregulation by TGFss in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. *PLoS One* 5: e13778
- Xue, L, Zhang, X, Li, Y, et al. (2011) Differences of immunophenotypic markers and signaling molecules between adenocarcinomas of gastric cardia and distal stomach. *Hum Pathol* 42: 594-601
- Zheng, L, Zhang, YL, Dai, YC, et al. (2017) Jianpi Qingchang decoction alleviates ulcerative colitis by inhibiting nuclear factor-kappaB activation. *World J Gastroenterol* 23: 1180-1188
- Zhou, H, Yu, X and Zhou, G (2017) NLRC5 silencing ameliorates cardiac fibrosis by inhibiting the TGFbeta1/Smad3 signaling pathway. *Mol Med Rep* 16: 3551-3556

Figures

Figure 1

The relative weight changes of rats in different groups. The data are presented as the mean $\pm$ SD. * $P < 0.05$, compared with control group; # $P < 0.05$, compared with PQ group.

Figure 2

The lung coefficient changes of rats in different groups. The data are presented as the mean $\pm$ SD. * $P < 0.05$, compared with control group; # $P < 0.05$, compared with PQ group.

Figure 3

H&E stained histological evaluation of rat lungs in the control, 5-ASA, PQ and PQ + 5-ASA groups on days 3, 7, 14, 28 after PQ intragastric administration (Control group and 5-ASA group: $\times 100$ magnification, PQ group and PQ+5-ASA group: $\times 200$ magnification). Compared with control group, PQ exposure showed progressively interstitial edema and inflammatory cell infiltration in the alveolar space and septum at day 3 and 7. Both 14 and 28 day of PQ group showed fibroblast proliferation, increase of collagen fibers and fibrous thickening of the alveolar walls. At the same time, PQ + 5-ASA groups showed remarkably decreased the pulmonary pathological damage of rats.

Figure 4

The effects of PQ on the Hydroxyproline (HYP) content in lung tissue of rats. Compared with PQ group, 5-ASA treatment could significantly decrease the HYP content in lung tissue of rats. All quantitative data are the mean $\pm$ SD. * $P < 0.05$, compared with control group. # $P < 0.05$, compared with PQ group.

Figure 5

Histological evaluation of rat lungs in the control, 5-ASA, PQ and PQ+5-ASA group on day 28 after PQ exposure with Masson stain (A and B:×400 magnification, C and D:×400 magnification). a: control group, b: 5-ASA group, c: PQ group, d: PQ+5-ASA group. Compared to control, PQ exposure increased fibrosis (seen as blue collagen deposition) in the alveolar regions and small bronchioles at day 28. The PQ+5-ASA group exhibited decreased collagen deposition in lung tissue of rats.

Figure 6

Treatment with 5-ASA decreases PQ-induced the expression of TGF- β 1 in lung tissue of rats ($\times 200$ magnification). The stainings of TGF- β 1 (brown in the cytoplasm: black arrowhead) were markedly down-regulated by PQ+5-ASA treatment for 14 d and 28 d after PQ intoxication.

Figure 7

Treatment with 5-ASA increase PQ-induced the expression of PPAR γ in lung tissue of rats($\times 200$ magnification). The stainings of PPAR γ (brown in the cell nucleus) were markedly up-regulated by PQ+5-ASA treatment for 3 d, 14 d and 28 d after PQ intoxication.

Figure 8

Treatment with 5-ASA significantly decrease the level of p-Smad3 in lung tissue of rats. Intensities of the immunoreactive bands were quantified by densitometric scanning. Values are means±SD. *P < 0.05, compared with control group. #P < 0.05, compared with PQ group.

Figure 9

5-ASA abolishes the PQ-induced activation of TGF- β 1 pathway signaling in human lung fibroblasts WI38VA13 cells. The WI38VA13 cells were pretreated with 10mM 5-ASA for 2 hr prior to 200 μ M PQ for 12, 24 and 48 hr. Treatment with 5-ASA significantly increased the level of PPAR γ (A), reduced the expression of TGF- β 1 (B) and the phosphorylation level of Smad3(C) as determined by Western blot. Intensities of the immunoreactive bands were quantified by densitometric scanning. Values are means±SD. *P < 0.05, compared with control group. #P < 0.05, compared with PQ group.