

Dipotassium Glycyrrhizinate Against Fibrotic Effects of Histamine Depletes Intracellular Glutathione to Discomfort Mitochondrial, Inducing Cell Apoptosis/Ferroptosis of Over-Activated Lung Fibroblast

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Abstract: Pulmonary fibrosis (PF) is the common outcome of a variety of chronic lung diseases and systemic diseases, and the consequence of Coronavirus Disease 2019 (COVID-19). There is currently a lack of effective treatments to reverse this disease or prevent its development. The purpose of this study is to investigate the effect of dipotassium glycyrrhizinate (DG) on lung fibroblasts and the possible mechanism of alleviating pulmonary fibrosis. Extract primary lung fibroblasts, establish cell models, and use time-lapse photography to continuously monitor the effects of dipotassium glycyrrhizinate and histamine on the morphology of lung fibroblasts. The content of glutathione in cells was detected by the fluorescence method. Cell mitochondrial membrane potential was examined, protein expressions of P53, Caspase-3, Bax and Bcl-2 of lung fibroblasts were evaluated by western blot and immune cytochemistry. Our data demonstrated that dipotassium glycyrrhizinate inhibited histamine-induced cell growth/proliferation, glutathione content, mitochondrial membrane potential; also down-regulated P53 and up-regulated the ratio of Bax/Bcl-2, leading to the ferritosis/apoptosis of activated lung fibroblasts, against the fibrotic effects of histamine. Present study provided a new pharmaceuticals mechanism of dipotassium glycyrrhizinate for the treatment of lung fibrosis that dipotassium glycyrrhizinate against fibrotic effects of histamine depletes intracellular glutathione to discomfort mitochondrial, inducing cell apoptosis/ferroptosis of over-activated lung fibroblast.

Keywords: Pulmonary Fibrosis, Dipotassium Glycyrrhizinate, Histamine, GSH, Inflammatory Injury

1. Introduction

Pulmonary fibrosis (PF) is the consequence of various types of severer lung diseases and systemic diseases, including Coronavirus Disease 2019 (COVID-19), progressive and destructive. Its main characteristics are alveolar epithelial cell damage, inflammatory cell infiltration, epithelial-mesenchymal transition, abnormal activation and proliferation of lung fibroblasts, and excessive deposition of extracellular matrix (ECM), eventually leading to substantial destruction of the lung [1].

Lung fibroblast is the primary cell for synthesizing the extracellular matrix in the lung, producing and releasing cytokines. Under the co-stimulation of various factors, lung fibroblast becomes activated, differentiated and proliferative, secreting a large amount of ECM components and increasing matrix deposition. During the cycles of pneumonia injury and healing, lung fibroblast plays a decisive role in the pathogenesis of pulmonary fibrosis [2].

Mast cell (MC) is a tissue type of immune-granulocyte, and plays an essential role in the occurrence and development of pulmonary fibrosis, mainly manifested as an increase in the number and activity of mast cells in lung tissue [3, 4]. It has been known that mast cell number is elevated [5] and positively correlated with lung fibroblast accumulation [6] in fibrotic lungs of the patients. The activation of mast cell releases an arrange of mediators including cytokines, proteases, and ECM components involved in fibrotic diseases and may drive fibroblast differentiation [7, 8].

Histamine is one of the critical mediators released by mast cell activation/degranulation with the essential physiological function by binding to its receptors [9], which is abundant in lung tissue [10]. Histamine induces the contraction of human lung fibroblast-mediated collagen gel via the H1 receptor [11]; and affects blood vessels strongly. Many studies on the expressions of histamine and its receptors

in pneumonia have suggested that histamine and its receptors may be involved in the process of pneumonia injury and healing.

Glutathione (GSH) is an important antioxidant in the body, and is synthesized in the cytoplasm and most distributed in the cytoplasm [12]. A part of GSH is transported into the mitochondria by transporters, regulating ROS balance. Decreased GSH concentration in cells or decreased GSH up-taken by mitochondria leads to a decrease in GSH levels in mitochondria, leading to oxidative stress and further tissue injury [13].

Glycyrrhizic acid (GA) is one of the essential active ingredients extracted from the root of licorice and has a strong non-specific antioxidant capacity [14]. Glycyrrhizic acid inhibits monocyte chemotactic protein 1 (MCP-1), tumor necrosis factor- α (TNF- α), and other pro-inflammatory factors through the nuclear factor- κ B (NF- κ B) signaling pathway, and regulates inflammation and oxidative stress-related reactions, effectively improving the acute inflammatory damage and fibrosis development of liver, kidney and other tissues [15, 16]. Structurally, two hydrogen ions in the structure of glycyrrhizic acid were replaced by two potassium ions respectively to form its derivative dipotassium glycyrrhizinate (DG), which improves the water solubility, and the efficacy remains unchanged.

This study was to investigate the effects of dipotassium glycyrrhizinate on histamine-induced lung fibroblasts, to explore the cellular mechanism of dipotassium glycyrrhizinate and the therapeutic potential on pulmonary fibrosis, to examine its anti-histamine function, aiming to provide a theoretical and therapeutic basis for its alleviating pulmonary fibrosis.

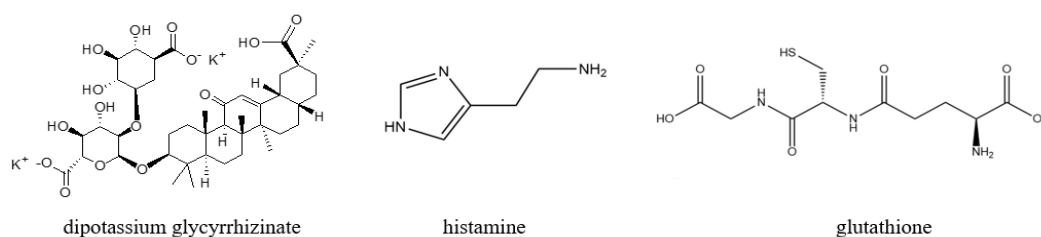


Figure 1: The chemical structure of dipotassium glycyrrhizinate and histamine.

2. Materials and Methods

2.1. Chemicals and Materials

Histamine was purchased from Aladdin, Loratadine (LTD), a histamine receptor antagonist was purchased from Aladdin, dipotassium glycyrrhizinate was purchased from Xi'an Biological Company (Xi'an, China). All cell culture materials were purchased from Gibco BRL (Grand Island, NY, USA). The antibodies (against P53, Caspase-3, Bax, Bcl-2, GAPDH; also anti-rabbit IgG-HRP, anti-goat IgG-HRP, and anti-mouse IgG-HRP) used were obtained from Santa Cruz Biotechnology (SCBT, Santa Cruz, CA, USA). Other chemicals were purchased from Sigma (St. Louis, MO, USA) or Aladdin Industrial (Shanghai, China). Otherwise, it would be stated.

2.2. Rat Primary Lung Fibroblasts Extraction and Culture

Rat primary lung fibroblasts were prepared as previously described [17]. Briefly, the lung tissues were separated from mice, and the connective tissues and adherent fat were removed. The lung tissues were rinsed three times with phosphate-buffered saline (PBS) containing 1% double-antibody and then cut into about 1 mm³ pieces and placed in completed DMEM containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 mg/mL streptomycin in a 5% CO₂ incubator at 37 °C until cells came out from tracheae tube pieces [18]. When a layer of fibroblasts grows on the bottom of the petri dish near the confluence, trypsinize the lung fibroblasts for 5 minutes and resuspend them in 10% fetal bovine serum (FBS), and resuspend them in 10% fetal bovine serum (FBS) Replant in the middle. We always use cells before the 20th generation in the experiments we describe below.

2.3. Cell Morphology and Time-Lapse Photography

The cells treated in various conditions were monitored continuously for up to 48 hours using time-lapse photography. The images were taken 0, 8, 24, 48 hours at the same position within well by a Leica X10 objective lens and the computer imaging software (Leica Application Suite).

2.4. The Levels of GSH in Lung Fibroblasts

Cells were seeded (8×10^3 cells/well) in a 96-well plate in completed DMEM and incubated overnight at 37 °C in 5% CO₂. histamine (0.1 mM), Loratadine (20 µg/mL), dipotassium glycyrrhizinate (30 mg/mL) or Loratadine and histamine (20 µg/mL and 0.1 mM), dipotassium glycyrrhizinate and histamine (30 mg/mL and 0.1 mM). After 24 h, After 24 hours, the cells were collected in a 96-well plate, and the content of glutathione in the cells was detected by the glutathione fluorescence method.

2.5. Measurement of Mitochondrial Membrane Potential ($\Delta\Psi_m$)

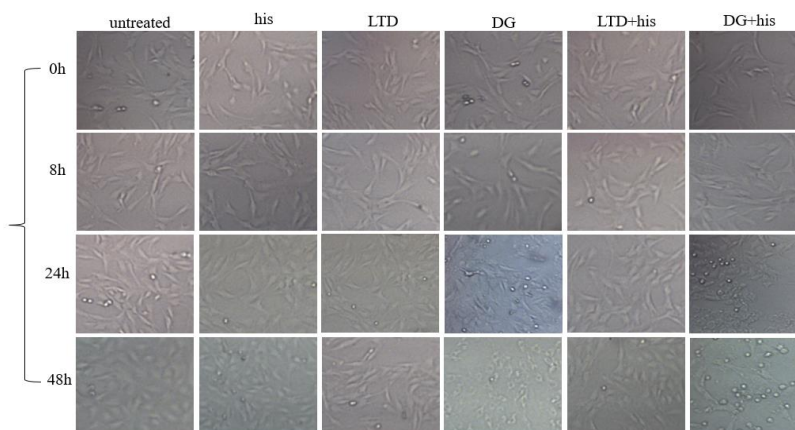
Mitochondrial membrane potential was measured by mitochondrial membrane potential assay kit with JC-1 (Beyotime, Nanjing, China), according to manufactory instruction. The changes in mitochondrial membrane potential were detected by the fluorescence inverted microscope to detect green and red fluorescence intensity.

2.6. Western Blot Assay

The protein contents of lung fibroblasts were determined using the BCA method. The equal proteins were subjected to separation by SDS-PAGE and transferred to polyvinylidene difluoride membranes; after being blocked with 5% FBS, followed by being blotted with primary antibodies against P53, Caspase-3, Bax, Bcl-2, GAPDH; then horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody. Enhanced chemiluminescence (Perkin Elmer, Waltham, USA) was to develop the signals. The images were captured using a ChemiDoc™ CRS + Molecular Imager (Bio-Rad Laboratories, USA) and quantified by Image Lab software (version 7.0, Bio-Rad Laboratories).

2.7. Statistical Analysis

Statistical analyses and graphs were performed using GraphPad Prism 7 package. The data were expressed as the mean \pm SEM for cell-model analysis, the Mann-Whitney non-parametric test was used to compare the differences between the cells in treatment conditions and untreated condition. * $p < 0.05$ was taken as statistically significant, ** $p < 0.01$, statistically very significant.



(A) Time-lapse photography: the images taken at different time point at same area (mid-zone) in the cell culture wells, lung fibroblasts were treated with different conditions of histamine (his, 0.1mM), loratadine (LTD, 20µg/mL), dipotassium glycyrrhizinate (DG,30mg/mL), loratadine mixed with histamine (LTD+his, 20µg/mL with 0.1mM), dipotassium glycyrrhizinate mixed with histamine (DG+his, 30mg/mL and 0.1 mM).

Figure 2: Dipotassium glycyrrhizinate, histamine influenced lung fibroblasts morphology.

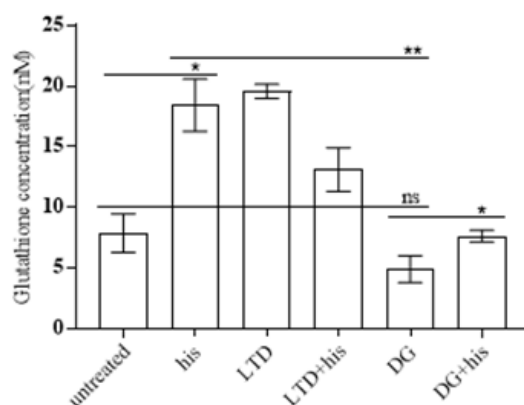
3. Results

3.1. Dipotassium Glycyrrhizinate, Histamine Influenced Lung Fibroblasts Morphology

The images from Time-lapse photography showed that dipotassium glycyrrhizinate inhibited cell growth and caused cell damage (Figure. 2) in a time-dependent manner compared with other conditions.

3.2. Dipotassium glycyrrhizinate reduce the level of glutathione induced by histamine in Lung Fibroblasts

The data of the glutathione fluorescence detection experiment show that compared with the untreated group, the intracellular glutathione content increased after treatment with histamine, the content decreased after treatment with histamine mixed with dipotassium glycyrrhizinate or LTD compared with the histamine group.

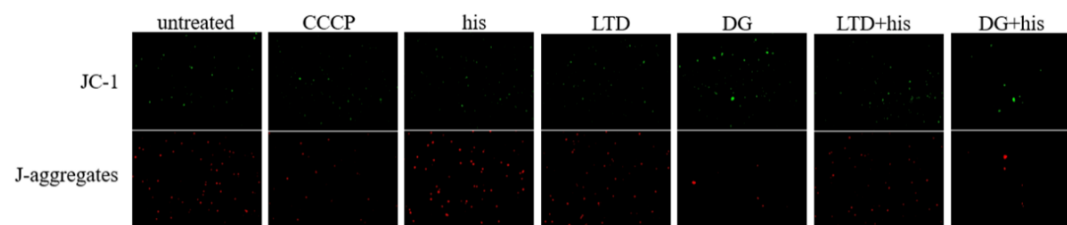


Cells were treated with histamine (his, 0.1 mM), loratadine (LTD, 20 μ g/mL), dipotassium glycyrrhizinate (DG, 30 mg/mL), loratadine mixed with histamine (LTD+his, 20 μ g/mL with 0.1mM), dipotassium glycyrrhizinate mixed with histamine (DG+his, 30mg/mL and 0.1mM) for 24h and the glutathione content in cells was detected by glutathione fluorescence assay. The fluorescence value (Ex365nm/Em420nm) was measured by a fluorescence spectrophotometer (mean \pm sem, n=6).

Figure 3: The contents of glutathione in lung fibroblasts in different conditions.

3.3. Dipotassium Glycyrrhizinate Can Recover the Increase in Mitochondrial Membrane Potential Induced by Histamine of Lung Fibroblasts

The data showed that histamine increased $\Delta\Psi_m$, loratadine or dipotassium glycyrrhizinate significantly reduced the $\Delta\Psi_m$ of lung fibroblasts, when dipotassium glycyrrhizinate mixed and histamine or loratadine mixed and histamine co-process lung fibroblasts, dipotassium glycyrrhizinate or loratadine against histamine effects and reduced the $\Delta\Psi_m$ of lung fibroblasts induced by histamine.

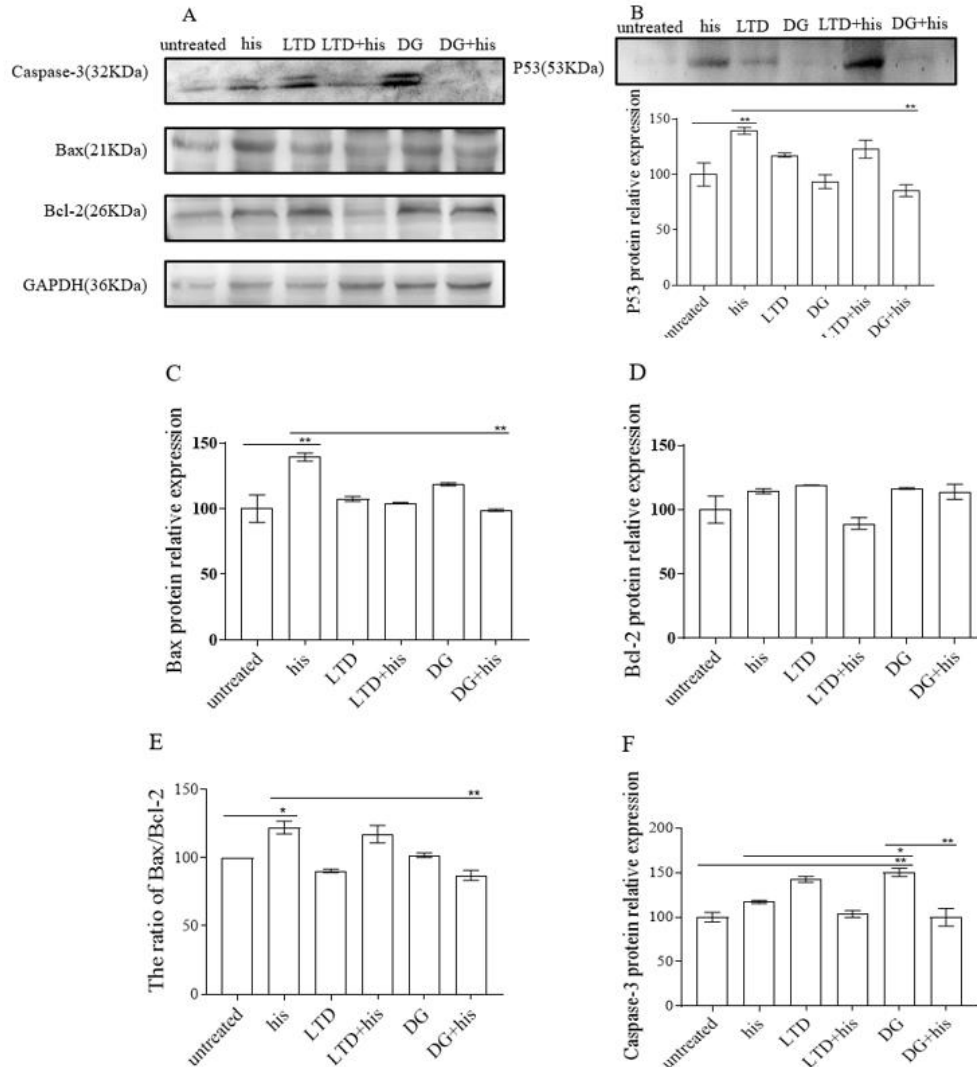


Mitochondrial Fluorescence was applied for lung fibroblasts untreated and treated with histamine (his, 0.1 mM), loratadine (LTD, 20 μ g/mL), dipotassium glycyrrhizinate (DG, 30mg/mL), loratadine mixed with histamine (LTD+his, 20 μ g/mL with 0.1mM), dipotassium glycyrrhizinate mixed with histamine (DG+his, 30mg/mL and 0.1mM) for 24h. Images were taken and combined using an immunofluorescence microscope (X40/X100) (Carl Zeiss, ZEN-3-0-blue-Hotfix-4).

Figure 4: Dipotassium glycyrrhizinate reduced the increase in mitochondrial membrane potential of lung fibroblasts induced by histamine.

3.4. Dipotassium Glycyrrhizinate Regulate the Expression of P53, Caspase-3, Bax, Bcl-2 in Lung Fibroblasts

The data from western blot showed that histamine increased the expression of P38, Caspase-3, Bax, Bcl-2 in lung fibroblasts, while dipotassium glycyrrhizinate significantly reduces the expression of these proteins.



The cell lysates extracted from the cells cultured with different conditions of histamine (his, 0.1mM), loratadine (LTD, 20µg/mL), dipotassium glycyrrhizinate (DG, 30mg/mL), loratadine mixed with histamine (LTD+his, 20 µg/mL with 0.1 mM), dipotassium glycyrrhizinate mixed with histamine (DG+his, 30mg/mL and 0.1 mM) for 24h. Western blotting and specific antibodies were applied to detect the protein expressions (n=3). The images were taken by Bio-Rad Laboratories and quantified by Image Lab software.

Figure 5: Dipotassium glycyrrhizinate regulate the expression of P53, Caspase-3, Bax, Bcl-2 in lung fibroblasts.

4. Discussion

Abnormal activation, differentiation, and proliferation of lung fibroblasts are the center processes in the development of lung fibrosis. Histamine, one of mast cell mediators, stimulates fibroblasts activation by binding to the histamine H1 receptor[9]. This study established an in-vitro cell model using freshly extracted rat primary lung fibroblasts and a histamine-induced cell activation model of primary lung fibroblast. Dipotassium glycyrrhizinate, loratadine (a histamine receptor antagonist used as the

positive control), histamine alone, and the mixture of dipotassium glycyrrhizinate with histamine were applied as the treatments.

Our data demonstrated that histamine stimulated lung fibroblasts activation, proliferation and migration, while dipotassium glycyrrhizinate inhibited the histamine induced-cell growth respectively (Fig. 2), which were associated with the changes in intracellular glutathione (GSH) content (Fig. 3) and the alterations of mitochondrial membrane potential (Fig. 4).

Mitochondria is in charge of oxidative metabolism in eukaryotes, and play a vital role in maintaining the stability of tissues and organs and the integrity of cell structure[19]. GSH is a regulator for ROS production and balance. The lower leveled GSH would increase ROS involved the permeability of the mitochondrial membrane, impairing the function of mitochondria, declining cell viability and inducing cell apoptosis or aggravating cell damage[20]. Intracellular loss of GSH is an early hallmark in the progression of cell death in response to different apoptotic stimuli[21, 22]. GSH is a strong reducing agent that can reduce the production of ROS and superoxide dismutase in the body and protect mitochondria[23]. Our study showed the fibrotic effects of histamine which can cause abnormal activation and proliferation of lung fibroblasts with increased cell viability, GSH level and mitochondria membrane potentials. While, potassium glycyrrhizinate reduced the histamine-induced glutathione content and mitochondrial membrane potential, leading to the increased ratio of Bax/Bcl-2 and caspase-3, that induces apoptosis of lung fibroblasts.

Recent studies have revealed a novel function of p53 in regulation of ferroptosis, a unique iron-dependent form of cell death driven by the accumulation of lipid-based reactive oxygen species (ROS) in cells[24]. Inducing apoptosis is one of the essential functions of P53. After activation, P53 can be used as a transcription factor to effectively induce cell apoptosis through the death receptor pathway and the mitochondrial apoptosis pathway[25]. The data showed that the expression of P53 in the cells were increased by histamine and decreased by dipotassium glycyrrhizinate, indicating that dipotassium glycyrrhizinate can recover the histamine-induced effects of lung fibroblasts (Fig. 5). In summary, dipotassium glycyrrhizinate induces apoptosis by reducing glutathione content and mitochondrial membrane potential ($\Delta\Psi_m$) in lung fibroblasts and regulating intracellular P53.

The decrease of mitochondrial membrane potential directly causes mitochondrial destruction, cell necrosis, and induces the activation of Bcl-2 and Caspase-3 families causes a cascade of cell apoptosis and leads to cell apoptosis. Activated caspase-3 is considered to be a marker of apoptosis[26]. Apoptosis is a form of active cell death regulated by genes, and its process is affected by many apoptosis-related genes such as the apoptosis-inhibiting gene Bcl-2 and the apoptosis-stimulating gene Bax[27]. Studies have confirmed that the lack of GSH in the cells can reduce the expression of Bcl-2 gene and aggravate the degree of apoptosis of tissue cells[28]. Intracellular GSH depletion enhanced Bax translocation, resulting in apoptosis[29]. Our experimental results indicate that dipotassium glycyrrhizinate reduced the content of glutathione and up-regulated the ratio of Bax/Bcl-2, Caspase-3 in over-activated/proliferative lung fibroblasts, inducing apoptosis of over-activated fibroblasts (Fig. 3 and Fig. 5).

In summery (Fig.6), this study provided new insight on cellular mechanisms of lung fibroblasts in the interaction with MC-histamine during the development of pulmonary fibrosis disease, also demonstrated the evidence that dipotassium glycyrrhizinate can reduce these histamine-induced effects on lung fibroblasts and promote ferroptosis/apoptosis of abnormally activated/proliferated lung fibroblasts to release the pulmonary fibrosis, implicating that dipotassium glycyrrhizinate against fibrotic effects of histamine depletes intracellular glutathione to discomfort mitochondrial, inducing cell apoptosis/ferroptosis of over-activated lung fibroblast, which needs attention, indeed, especially for current COVID-19 treatment.

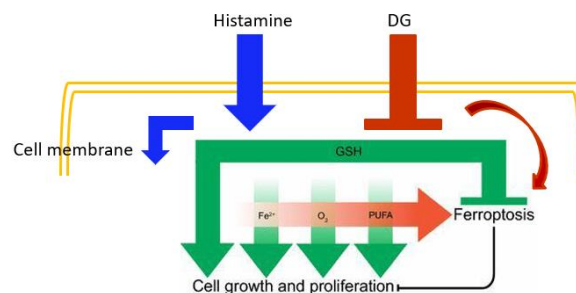


Figure 6: The mechanism of dipotassium glycyrrhizinate (DG) against histamine fibrotic effects on glutathione (GSH) of lung fibroblasts, leading to cell ferroptosis/apoptosis.

Conflict of interest

The authors have declared that no conflict of interest exists.

Acknowledgements

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References

- [1] D.N. Cook, D.M. Brass, D.A. Schwartz, A matrix for new ideas in pulmonary fibrosis, *Am J Resp Cell Mol Biol*, 27 (2002) 122-124. <https://doi.org/10.1165/ajrcmb.27.2.f245>.
- [2] Y. Zhang, H. Jiao, Y. Wu, et al., P120-catenin regulates pulmonary fibrosis and TGF-beta induced lung fibroblast differentiation, *Life Sciences*, 230 (2019) 35-44. <https://doi.org/10.1016/j.lfs.2019.05.052>.
- [3] M. Wygrecka, B.D. Dahal, D. Kosanovic, et al., Mast Cells and Fibroblasts Work in Concert to Aggravate Pulmonary Fibrosis: Role of Transmembrane SCF and the PAR-2/PKC- α /Raf-1/p44/42 Signaling Pathway, *American Journal of Pathology*, 182 (2013) 2094-2108.
- [4] E.P. Moiseeva, K.M. Roach, M.L. Leyland, et al., CADM1 is a key receptor mediating human mast cell adhesion to human lung fibroblasts and airway smooth muscle cells, *PLoS One*, 8 (2013) e61579. <https://doi.org/10.1371/journal.pone.0061579>.
- [5] C. Shimbori, C. Upagupta, P.-S. Bellaye, et al., Mechanical stress-induced mast cell degranulation activates TGF- β 1 signalling pathway in pulmonary fibrosis, *Thorax*, 0 (2019) 1-11. <https://doi.org/10.1136/thoraxjnl-2018-211516>.
- [6] C.K. Andersson, A. Andersson Sjöland, M. Mori, Activated MCTC mast cells infiltrate diseased lung areas in cystic fibrosis and idiopathic pulmonary fibrosis, *Respiratory Research*, 12(139) (2011) 1-13. <https://doi.org/10.1186/1465-9921-12-139>.
- [7] C. Overed-Sayer, L. Rapley, T. Mustelin, et al., Are mast cells instrumental for fibrotic diseases?, *Frontiers in Pharmacology*, 4 (2013) 174. <https://doi.org/10.3389/fphar.2013.00174>.
- [8] T. YAMAMOTO, K. HARTMANN, B. ECKES, Mast cells enhance contraction of three-dimensional collagen lattices by fibroblasts by cell-cell interaction: role of stem cell factor/c-kit, *Immunology*, 99(3) (2000) 435-439. <https://doi.org/10.1046/j.1365-2567.2000.00973.x>.
- [9] F.E.R. Simons, K.J. Simons, Histamine and H1-antihistamines: celebrating a century of progress, *Clinical Reviews in Allergy & Immunology*, 128 (2011) 1139-1150. <https://doi.org/10.1016/j.jaci.2011.09.005>.
- [10] E. Veglia, A. Pini, A. Moggio, et al., Histamine type 1-receptor activation by low dose of histamine undermines human glomerular slit diaphragm integrity, *Pharmacological Research*, 114 (2016) 27-38.
- [11] M. Horie, A. Saito, Y. Yamauchi, et al., Histamine induces human lung fibroblast-mediated collagen gel contraction via histamine H1 receptor, *Experimental Lung Research*, 40(5) (2014) 222-236. <https://doi.org/10.3109/01902148.2014.900155>.
- [12] M. Qiu, F. Shi, F. Dai, et al., A reactive oxygen species activation mechanism contributes to Sophoridine-induced apoptosis in rat liver BRL-3A cells, *Journal of Ethnopharmacology*, 213 (2018) 376-383. <https://doi.org/10.1016/j.jep.2017.10.030>.
- [13] J.C. Fernández-Checa, A. Colell, C. Garc ía-Ruiz, S -Adenosyl- L -methionine and mitochondrial reduced glutathione depletion in alcoholic liver disease, *Alcohol*, 27 (2002) 179-183.
- [14] V.J. Thannickal, G.B. Toews, E.S. White, et al., Mechanisms of Pulmonary Fibrosis, *Annual Review of Medicine*, 55 (2004) 395-417. <https://doi.org/10.1146/annurev.med.55.091902.103810>.
- [15] H. Zhao, Z. Liu, H. Shen, et al., Glycyrrhizic acid pretreatment prevents sepsis-induced acute kidney injury via suppressing inflammation, apoptosis and oxidative stress, *European Journal of Pharmacology*, 781 (2016) 92-99. <https://doi.org/10.1016/j.ejphar.2016.04.006>.
- [16] H. Zhao, M. Zhao, Y. Wang, et al., Glycyrrhizic Acid Attenuates Sepsis-Induced Acute Kidney Injury by Inhibiting NF- κ B Signaling Pathway, *Evidence-Based Complementary and Alternative Medicine*, (2016). <https://doi.org/10.1155/2016/8219287>.
- [17] T. Wei, L. Liu, X. Zhou, Cortex Dictamnii extracts inhibit over-proliferation and migration of rat airway smooth muscle cells via FAK/p38/Bcl-2 signaling pathway, *Biomedicine & pharmacotherapy*, 102 (2018). <https://doi.org/10.1016/j.biopha.2018.03.039>.

- [18] I. Park, J. Shin, H. Jung, et al., Stimulatory effects of histamine on migration of nasal fibroblasts, *International Forum of Allergy & Rhinology*, 5(10) (2015) 923-928. <https://doi.org/10.1002/alr.21555>.
- [19] E. Łowicka, J. Bełowski, Hydrogen sulfide (H₂S) – the third gas of interest for pharmacologists, *Pharmacological Reports*, 59 (2007) 4-24.
- [20] C. Picard, J.-L. Casanova, A. Puel, Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IkappaBalpha deficiency, *Clin Microbiol Rev*, 24(2011) 490–497. <https://doi.org/10.1128/CMR.00001-11>.
- [21] C.L. Hammond, M.S. Madejczyk, N. Ballatori, Activation of plasma membrane reduced glutathione transport in death receptor apoptosis of HepG2 cells, *Toxicol Appl Pharmacol*, 195 (2004) 12-22. <https://doi.org/10.1016/j.taap.2003.10.008>.
- [22] R. Franco, J.A. Cidlowski, SLCO/OATP-like transport of glutathione in FasL-induced apoptosis: glutathione efflux is coupled to an organic anion exchange and is necessary for the progression of the execution phase of apoptosis, *J Biol Chem*, 281 (2006) 29542-29557. <https://doi.org/10.1074/jbc.M602500200>.
- [23] R.C. Francis, K. Vaporidi, K.D. Bloch, et al., Protective and Detrimental Effects of Sodium Sulfide and Hydrogen Sulfide in Murine Ventilator-induced Lung Injury, *Anesthesiology*, 115 (2011) 1012-1021. <https://doi.org/10.1097/ALN.0b013e31823306cf>.
- [24] S.J. Dixon, K.M. Lemberg, M.R. Lamprecht, et al., Ferroptosis: an iron-dependent form of nonapoptotic cell death, *Cell*, 149 (2012) 1060-1072. <https://doi.org/10.1016/j.cell.2012.03.042>.
- [25] J.D. Amaral, J.M. Xavier, C.J. Steer, et al., The Role of p53 in Apoptosis, *Discovery Medicine*, 45 (2010) 145-152.
- [26] S. Shin, B.J. Sung, Y.S. Cho, et al., An anti-apoptotic protein human survivin is a direct inhibitor of caspase-3 and -7, *Biochemistry*, 40 (2001) 1117-1123.
- [27] S.-M. Li, S.-K. Yao, N. Yamamura, et al., Expression of Bcl-2 and Bax in extrahepatic biliary tract carcinoma and dysplasia, *World Journal of Gastroenterology*, 9 (2003) 2579-2582.
- [28] A. Celli, F.G. Que, G.J. Gores, et al., Glutathione depletion is associated with decreased Bcl-2 expression and increased apoptosis in cholangiocytes, *The American Physiological Society*, 38 (1998) 749-747.
- [29] T. Honda, S. Coppola, L. Ghibelli, et al., GSH depletion enhances adenoviral bax-induced apoptosis in lung cancer cells, *Cancer Gene Therapy*, 11 (2004) 249-255. <https://doi.org/10.1038/sj.cgt.7700684>.

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PF: pulmonary fibrosis

ECM: extracellular matrix

MC: mast cell

his: Histamine

DG: dipotassium glycyrrhizinate

GA: glycyrrhizic acid

GSH: glutathione

MCP-1: monocyte chemotactic protein 1

TNF- α : tumor necrosis factor- α

NF- κ B: nuclear factor kappa-B

LTD: loratadine

FBS: fetal bovine serum

PBS: phosphate-buffered saline

HRP: horseradish peroxidase

SF: serum-free

DMSO: Dimethyl sulfoxide

PI: Propidium iodide