Montelukast effect on Clara Cell secretory protein (CC16) and Krebs von den Lungen-6(KL-6)in pulmonary fibrosis rats model

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Abstract

Pulmonary fibrosis(PF) is a condition in which the lungs became scarred and breathing becomes difficult. The most common type is idiopathic pulmonary fibrosis (IPF) which occurred to an unknown cause.PF pathogenesis passes through three stages the first one is an injury of alveolar cells followed by the inflammation stage then the repair stage. Bleomycin causes pulmonary fibrosis due to a lack of deactivating enzymes bleomycin hydrolase. Administration of bleomycinintratracheally causes injury of alveolar cells type II causing the release of inflammatory markers such as Interleukins (as IL-4) and transforming growth factor beta(TGFβ1) which cause conversion of fibroblast to myofibroblast that finally leads to fibrosis. Pirfenidone is an antifibrotic agent, that has anti-inflammatory and antioxidant effects by inhibition of TGFβ1. Montelukast is Cysteinyl leukotrienes (CysLT) antagonist bind to corresponding CysLT receptors like CysLT type-1 receptors located on respiratory airway smooth muscle cells, airway macrophages, and on various pro-inflammatory cells like eosinophils and some specific myeloid stem cells activities causing anti-inflammatory effect by inhibition of inflammatory markers as TGFβ1. In this study, we investigate the impact and mechanisms of montelukast on the pulmonary fibrosis rats model. Rats were divided into five groups, the control group received distilled water by gastric gavage for 28 days, the induction group received bleomycinintratracheally as a single dose, pirfenidone group received pirfenidone 50mg/kg for 28 days after 14 days from bleomycin dose, montelukast group was received montelukast 20mg/kg for 28 days after 14 days from bleomycin dose and the combination group received a half dose of pirfenidone and montelukast for 28 days after 14 days of bleomycin dose. After twenty-eight days from the treatment with montelukast or pirfenidone sacrifice rats and collect the blood samples from each group and compare the results by measuring the biomarkers [cub cell secretory protein 16 (CC16), Krebs von den lungen-6 (KL-6), platelet-derived growth factor (PDGF), IL-4 and TGFβ1]. We observed that the induction group elevated the level of all these markers after 14 days from bleomycin dose but pirfenidone, montelukast, and combination decreased the level of these markers after 28 days of treatment. In conclusion,montelukast has anti-inflammatory and antifibrotic effects by decreasing the level of inflammatory markers and TGFβ1 which is a key cytokine responsible for pulmonary fibrosis.

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Introduction

Pulmonary fibrosis(PF) is the result of lung damage and becoming scarred. There are several types of lung fibrosis including

asbestosis, COVID-19-related pulmonary fibrosis, drugs-induced pulmonary fibrosis, and idiopathic pulmonary fibrosis. The most common type is idiopathic pulmonary fibrosis[1]. pulmonary fibrosis is caused by many risk factors such as genetic factors (especially mucin alteration MUC5B)[2], autoimmune diseases(sclerosis, systemic lupus erythematosus, and ankylosing spondylitis)[3],cigarette smoking[4], aging[5], occupational and environmental factors[6], infectious agents (especially bacteria,fungal and viruses such as COVID19)[7], and drug-induced lung fibrosis(as bleomycin caused lung fibrosis due to deficiency of bleomycin hydrolase enzyme)[8]. The pathophysiology of lung fibrosis includes three stages starting with the first stage including injury of pneumocytes destroying the homeostasis of the airway epithelium thus decreasing the level of Clara cell secretory protein 16 (CC16) which has a protective role in maintaining the homeostasis of the airway epithelium and also has an anti-inflammatory effect[9] followed by the second stage causing inflammation and release of inflammatory markers such as interleukins and growth factors finally the third stage includes failure of the repair of injury[10]. Pirfenidone and nintedanib are two antifibrotic drugs which approved for the treatment of PF bythe European Medicines Agency (EMA) in 2011, followed by The US Food and Drug Administration (FDA) approval in 2014[11] where pirfenidone available in an oral dosage form which inhibits the production and release of pro-fibrotic and pro-inflammatory cytokines such as interleukins (as IL_6 and IL_4) and growth factors as transforming growth factor (TGF-β) and platelet-derived growth factor(PDGF) causing inhibit proliferation of fibroblast,collagen deposition and conversion of fibroblast to myofibroblast.

Montelukast is Cysteinyl leukotrienes (CysLT) antagonist that binds to corresponding CysLT receptors like CysLT type-1 receptors located on respiratory airway smooth muscle cells, airway macrophages, and on various pro-inflammatory cells like eosinophils and some specific myeloid stem cells activities causing anti-inflammatory effect by inhibition of inflammatory markers. Montelukastbinds with high affinity and selectivity to the CysLT type 1 receptor, which consequently assists in inhibiting any physiological actions of CysLTs like LTC4, LTD4, and LTE4 at the receptor that may facilitate the interstitial lung diseases. Montelukast also has an anti-inflammatory effect by modulating the level of TGFβ1[12].

Aim of study

Evaluate the effect of montelukast on CC16, KL-6,TGFβ1,PDGF, and IL-4 in bleomycin-induced lung fibrosis rat model

Materials and methods

Materials include drugs(bleomycin 15 I.U. celon labs india, pirfenidone 200mg Cipla India, montelukast 10mg denk-pharma Germany,Ketamine (as HCl)vial for injection 100mg/ml kepro Holland, andXylasin (as HCl) vial for injection 20mg/ml), solvents(DimethysulfoxideACSSpain and Distilled waterPioneerIraq), kits (Transforming growth factor beta-1 (TGF B1)MyBioSourceUSA, Platelet-derived growth factorMyoBiosourceUSA, Club cell secretory protein16 (CC16)MyoBiosourceUSA, Krebs von den lungen-6MyoBiosourceUSA and Interleukin-4MyoBiosourceUSA where the assessment of these biomarkers is based on sandwich ELISA technology.

SixtyWistar albino male rats were used in this. They were handled according to the ethics committee in the College of Pharmacy/Mustansiriyah University. The rats were placed in big cages with free reach to food and water. The rats were divided into five groups Group (I) control group: included 12 rats who received distal water by gastric gavage once every day for 28 days.Group(II) induction group: Include 12 rats who received a single dose of bleomycin 8.3 I.U./ as sulfate salt dissolved in 0.1 ml of normal saline intratracheally.Group (III) standard treatment group: include 12 rats who received bleomycin (8.3 U/kg) as sulfate salt dissolved in 0.1 ml of normal saline intratracheally at day 0 and treated with 50mg/kg pirfenidone dissolved with dimethyl sulfoxide 10% per day orally for 28 days.Group (IV) treatment group: include 12 rats who received bleomycin (8.3 U/kg) as sulfate salt dissolved in 0.1 ml of normal saline intratracheally and treated with 20mg/kg montelukast dissolved in distilled water per day orally for 28 days after bleomycin dose.Group (V)combination group: include 12 rats who received bleomycin (8.3 U/kg) as sulfate salt dissolved in 0.1 ml of normal saline intratracheally and treated with a half dose of montelukast 10mg/kg per day plus a half dose of pirfenidone 25mg/kg per day orally for 28 days.Aftertwenty-eight days from the treatment with montelukast or pirfenidone sacrifice rats and collect the blood samples from each group and compare the results.

Methods include assessment of biomarkers CC16, KL-6, TGFβ, PDGF, and IL-4by sandwich ELISA technology.

Result

The descriptive statistics were done by ANOVA test for serum CC16 concentration which is represented as mean ± SD (table 3-1), after twenty-eight days there was a highly significant difference in the induction group (15.08±2.17) when compared with the control group (29.08±6.91) *p<0.01;* but The serum level of CC16 was highly significant in pirfenidone group (72.84±5.60), montelukast group (77.15±1.50), and the combination group(72.15±1.50)in comparison with the induction group (15.08±2.17) *P<0.01*after twenty-eight days from treatment as shown in the table(1).

The serum KL-6 concentration showed a significant difference in the induction group (737.19±37.26) in comparison with the control group (144.74±13.12) *P<0.05*, but the serum levels of KL-6 were a highly significant difference in pirfenidone group (644.17±25.16), montelukast group (658.15±19.89), and the combination group(659.04±22.12) when compared with the induction group (737.19±37.26) *P<0.01* after twenty-eight days from treatment as shown in the table(1).

TABLE(1) explainmean±std. deviation of all biomarkers measured in this study

Biomarkers	Control group	Induction group	Pirfenidone group	Montelukast group	Combination group
TGF-β	135.12±12.11	274.82 ±4.86	120.88±8.17	240.32±1.53	167.72±2.59
IL-4	81.47±14.84	178.94±4.76	90.83±5.48	124.88±12.5	98.66±13.05
PDGF	15.40±1.24	78.17±4.12	71.43±3.93	71.61±6.64	54.34±4.01
CC16	29.08±6.91	15.08±2.17	72.84±5.60	77.15±1.50	72.15±1.50
KL-6	144.74±13.12	737.19±37.26	644.17±25.16	658.15±19.89	659.04±22.12

The serum PDGF showed a significant increase in the induction group (78.17±4.12) in comparison with the control group (15.40±1.24) *P<0.05*,but the serum levels of PDGF were no

significant difference for pirfenidone group(71.43±3.93), and the montelukastgroup(71.61±6.64) when compared with the induction group(78.17±4.12)*P=0.919,and P=0.927* respectively;but the combination group(54.34±4.01) showed a significant decline when compared with the induction group (78.17±4.12) *P<0.05*after twenty-eight days from treatment as shown in the table(1).

The serum TGF-β1 concentration showed after twenty-eight days a very highly significant elevation in the induction group(274.82 ±4.86) in comparison with the control group (135.33±12.11)*P<0.01*. The serum levels of TGF-B1 also were a very highly significant difference for the pirfenidone group(120.88±8.17), montelukast group (240.32±1.53), and the combination group(167.72±7.32) when compared with the induction group(203.15±3.39) *P<0.01*after twenty-eight days from treatment as shown in the table(1).

The serum IL-4 concentration showed a very highly significant elevation in the induction group(178.94 ±4.76) in comparison with the control group (81.47±14.84) *P<0.01*, but the serum levels of IL-4 were a very highly significant difference for pirfenidone group(90.83±5.48), montelukast group (124.88±12.51), and combination group(98.66±13.05) when compared with the induction group(178.94±4.76) *p<0.01*after twenty-eight days from treatment as shown in table(1).

Discussion

Pulmonary fibrosis is a condition in which the lung parenchyma will be scarred[13]. Numerous studies and clinical trials showed that montelukast has a positive effect on both humans and animals regarding the treatment of pulmonary fibrosis by lowering inflammatory biomarkers[14].

For that,it was chosen to study the impact of montelukast on the inflammatory biomarkers, including CC16, KL6, PDGF, TGF-β1, and IL-4, that are involved directly in the inflammatory phase of pulmonary fibrosis and compare the results to a well-known antifibrotic medication (Pirfenidone) used to treat pulmonary fibrosis[11].

In the impact of montelukast on CC16**,** this study reveals that the induction group significantly decrease the level of CC16 in serum after twenty-eight days from the induction of pulmonary fibrosis and this result came in agreement with[15].In pirfenidone group, show a significant elevation in CC16 after twenty-eight days from the onset of the treatment, this result is confirmed by [16].Montelukast treatment and the combination group resulted in a highly significant elevation in the levels of cc16 twenty-eight days from the onset of the treatment, this result came in agreement with [17].

In the impact of Montelukast on KL-6,this study explains that the induction group significantly increased the level of KL-6 in serum after twenty-eight days from the induction of pulmonary fibrosis and this was confirmed by[18]**.** Pirfenidone showsa significant decline in KL-6 in serum after twenty-eight days from the onset of the treatment and this was confirmed by[19].In montelukast and the combination of montelukast and pirfenidone, a highly significant decrease in the serum level after twenty-eight days from the onset of the treatment and these results came in agreement with[20]

In the impact of Montelukast on PDGF,this study reveals that the induction group significantly increased the level of PDGF after twenty-eight days from the induction of pulmonary fibrosis this is confirmed by[21]**.**Pirfenidone group, after twenty-eight days from the onset of the treatment, show no significant difference in serum levels of PDGF this was confirmed by [22], althoughMontelukast and combination groups

showed a significant decrease in the level of PDGFafter twenty-eight days from the onset of the treatment and this came in agreement with [23].

In the impact of Montelukast on TGFβ1,this study reveals that the induction group highly significantly increased the level of TGF-β1 in serum after twenty-eight days from the induction of pulmonary fibrosis this is confirmed by [24].In pirfenidone group, the level of TGFβ1**was** show a highly significant decline this was confirmed by [22].Montelukast and the combination groups show a highly significant decline in TGF-β1 level of serum after twenty-eight days from the onset of the treatment these results came in agreement with[25].

In the impact of Montelukast on IL-4, this study reveals that the induction group highly significantly increased the level of IL-4 in serum after twenty-eight days from the induction of pulmonary fibrosis this is confirmed by [26].In pirfenidone, the serum levels of IL-4 were very highly significant after twenty-eight days from the onset of the treatment and this is confirmed by [27].Montelukast and the combination groups show a highly significant decline in IL-4 levels in serum levels after twenty-eight daysfrom the onset of the treatment and this result came in agreement with [28].

Conclusion

Montelukast has a protective effect against bleomycin-induced pulmonary fibrosis by decreasing the level of inflammatory markers especially TGF-B1 which play an important role in pulmonary fibrosis. Montelukast has an anti-inflammatory role in the decline of growth factors and IL-4 finally, Pirfenidone and montelukast have a significant combinative effect against pulmonary fibrosis after twenty-eight days of treatment.

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