Histopathological evaluation of induced pulmonary fibrosis under the effect of montelukast

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Abstract:

Pulmonary fibrosis (PF) is an interstitial lung disease leading to scarring of the lung. There are several types of lung fibrosis as familial pulmonary fibrosis, idiopathic pulmonary fibrosis, and others associated with non-specific interstitial pneumonia. The most common type is idiopathic pulmonary fibrosis which is an unknown cause. Lung fibrosis causes changes in the histology of the lung by the disappearance of the lung parenchyma, replaced by an inflammatory infiltrate, and mild thickening of the pulmonary artery. The management of pulmonary fibrosis included Azathioprine, corticosteroid, and N-acetyl cysteinyl in 2011 but in 2014 this guideline was removed and replaced by nintedanib and pirfenidone. This study used Pirfenidone, as standard therapy for the treatment of pulmonary fibrosis, and montelukast is Cysteinyl leukotrienes (CysLT) antagonist which binds to its receptor (CysLTE4) located on smooth muscle cells of the respiratory airway causing anti-inflammatory effect by inhibition of inflammatory markers as TGFβ1. Sixty male rats were divided into five groups,12 rats for each group where the control group received distilled water by gastric gavage, the induction group received bleomycin intratracheally as a single dose, the pirfenidone group received pirfenidone 50mg/kg, montelukast group received montelukast 20mg/kg and the combination group received a half dose of pirfenidone and montelukast. After twenty-eight days after the treatment with montelukast or pirfenidone sacrifice rats and collect the organ (lungs) from each group were then placed in buffer formalin 10% for histopathological study. After 14 days from bleomycin dose, results show that bleomycin cause massive disappearance of pulmonary parenchyma that was replaced by an inflammatory infiltrate and medial thickening of the pulmonary artery in all groups, but montelukast and pirfenidone show normal lung paranchyma and pulmonary artery after 28 days of treatment in pirfenidone, montelukast, and combination groups.
In conclusion, that bleomycin changes the histology of the lung causing induction of lung fibrosis in all groups after 14 days except control group but pirfenidone, montelukast, and combination of half dose of pirfenidone with a half dose of montelukast return the lung to normal architecture after 28 days of treatment.
Key words: pulmonary fibrosis, materials and methods, results, and discussion.

التقييم النسيجي المرضي للتليف الرئوي المحفز تحت تأثير علاج المونتلوكست

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الخلاصة:
التليف الرئوي هو مرض خلالي في الرئة يؤدي إلى تندب الرئة. هناك عدة أنواع من التليف الرئوي مثل التليف الرئوي العائلي ، والتليف الرئوي مجهول السبب ، وأنواع أخرى مرتبطة بالالتهاب الرئوي الخلالي غير النوعي. النوع الأكثر شيوعًا هو التليف الرئوي مجهول السبب وهو سبب غير معروف. علاج التليف الرئوي يشمل الايزوثيوبرين, كورتيكوستيرود, و ان استل سستين في 2011 ولكن في 2014 تم استخدام البرفندون والنتندانيب كعلاج لتليف الرئة. يسبب تليف الرئة تغيرات في أنسجة الرئة عن طريق اختفاء نسيج الرئة الاسفنجي واستبداله بالارتشاح الالتهابي ، تثخن في الشريان الرئوي. استخدمت هذه الدراسة علاج البرفندون لعلاج التليف الرئوي وعلاج المونتلوكست وهو مضاد للستنيل ليكوترين والذي يرتبط ب سستنيل ليكوترين اي 4 الموجودة على خلايا العضلات الملساء في مجرى الهواء التنفسي مما يسبب تأثيرًا مضادًا للالتهابات عن طريق تثبيط العلامات .TGFβ1
تم تقسيم ستين من ذكور الجرذان إلى خمس مجموعات ، 12 فأرًا لكل مجموعة حيث تلقت المجموعة الضابطة الماء المقطر بالتزقيم المعدي ، تلقت المجموعة الحثية بلوميسين داخل الرغامى كجرعة وحيدة ، تلقت مجموعة البيرفينيدون بيرفينيدون 50 مجم / كجم ، تلقت مجموعة مونتيلوكاست مونتيلوكاست 20 مجم / كجم والمجموعة المركبة التي تلقت نصف جرعة من بيرفينيدون ومونتيلوكاست. بعد ثمانية وعشرين يومًا بعد العلاج بالمونتيلوكاست أو البيرفينيدون ، تم وضع الفئران التي جمعت العضو (الرئتين) من كل مجموعة في محلول فورمالين عازل بنسبة 10٪ لدراسة التشريح المرضي. بعد 14 يومًا من جرعة البليوميسين ، يُظهر أن البليوميسين يسبب اختفاءًا هائلاً للحمة الرئوية التي تم استبدالها بالترشح الالتهابي والسماكة الإنسي للشريان الرئوي في جميع المجموعات ، لكن المونتيلوكاست والبيرفينيدون يظهران بارانكيما رئويًا طبيعيًا والشريان الرئوي بعد 28 يومًا من العلاج في بيرفينيدون ، ومونتيلوكاست ، ومجموعات مختلطة..
في الختام ، فإن البليوميسين يغير نسيج الرئة مما يؤدي إلى تحريض تليف الرئة في جميع المجموعات التي تلقت البليوميسين بعد 14 يومًا من جرعة البليوميسين ولكن بيرفينيدون ومونتيلوكاست ومزيج من نصف جرعة من بيرفينيدون مع نصف جرعة مونتيلوكاست يعيد الرئة إلى العمارة الطبيعية بعد 28 يومًا من العلاج.

الكلمات المفتاحية: تليف الرئة , طرق العمل , نتائج و التفسير

Introduction
Pulmonary fibrosis is meaning the thickening of the parenchymal tissue of the lungs. There are several types of pulmonary fibrosis but the most common is idiopathic pulmonary fibrosis which is an unknown cause. Pulmonary fibrosis is caused by several factors such as age-related pulmonary fibrosis drug-induced lung fibrosis(such as bleomycin), smoking, infectious agents (such as COVID-19), asbestos, genetic factors, and autoimmune diseases [1]. The pathogenesis started with the injury of pneumocytes type II and then releasing of inflammatory mediators and finally the impaired repair process [2]. Pirfenidone and nintedanib are two drugs approved in 2011 by the European Medicines Agency (EMA) and then approved in 2014 by The Food and Drug Administration (FDA) for the treatment of pulmonary fibrosis[3]. Pirfenidone is an antifibrotic agent which approved for treatment of IPF [4]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 factors (TGF-β) and platelet-derived growth factor(PDGF) causing inhibit proliferation of fibroblast, collagen deposition and conversion of fibroblast to myofibroblast [5]. Nintedanib competitively inhibited the kinase activity of various receptors including receptors tyrosine kinases(RTKs) and non-receptor tyrosine kinases(nRTKs) where RTKs include vascular endothelial growth factor receptors(VEGFRs), platelets derived growth factor receptors(PDGFRs) and fibroblast growth factor receptors(FGFRs) [6]. These receptors are responsible for angiogenesis, proliferation, and fibroblast proliferation and migration in the lung. Nintedanib reversibly and competitively

blocked the intracellular signaling pathways that are essential for proliferation, migration, and conversion of fibroblast to myofibroblast which causes fibrosis by inhibiting the adenosine triphosphate (ATP) binding pocket of VEGFR, PDGFR, and FGFR [7]

Montelukast is a Cysteinyl Leukotrienes (CysLT) Antagonist that binds to specific CysLT receptors, such as CysLT type-1 receptors found on smooth muscle cells of the respiratory airway, airway macrophages, and various pro-inflammatory cells [8], such as eosinophils and some specific myeloid stem cells, to produce an anti-inflammatory effect by inhibiting the production of inflammatory markers [9] thus inhibit the symptoms of asthma and other interstitial lung diseases from this explain that all physiological effects of CysLTs were inhibited by montelukast thus montelukast has an anti-inflammatory effect [10]

Aim of study

To evaluate the effect of montelukast on tissue histology in bleomycin-induced pulmonary fibrosis rat's 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, and Xylasin (as HCl) vial for injection 20mg/ml), solvents (Dimethysulfoxide ACS Spain and Distilled water Pioneer Iraq).

A-Preliminary study

Eighteen Wistar albino rat males were used in a pilot study to obtain the effective dose for montelukast. The rats were divided into four groups each group with six rats receiving different doses of montelukast (5mg/kg, 10mg/kg, and 20mg/kg) after induction of pulmonary fibrosis by bleomycin, and the fourth group was the control group that received distilled water (D.W.) in equivalent volume that dissolves of montelukast.

B- Main study

Sixty Wistar 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 bleomycin intratracheally {model for induction of pulmonary fibrosis} as in Mustansiriyah university/Collage of pharmacy and a previous study [11] [12] (8.3 I.U/kg) in a single dose. Group (III) standard treatment group: include 12 rats who received bleomycin intratracheally (8.3 I.U/kg) in a single dose at day 0 and treated with 50mg/kg pirfenidone dissolved with dimethyl sulfoxide 10% per day orally for 28 days after 14 days from bleomycin dose as in previous study [13]. Group (IV) treatment group: include 12 rats who received bleomycin intratracheally (8.3 I.U/kg) in a single dose and treated with 20mg/kg montelukast dissolved in distilled water per day orally for 28 days after 14 days from bleomycin dose as in previous study [13]. Group (V)combination group: include 12 rats who received bleomycin intratracheally (8.3 I. U/kg) in a single dose 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 after 14 days from bleomycin dose. After twenty-eight days from the treatment with montelukast or pirfenidone sacrifice rats and collect the orgens (lung) from each group for histopathological study and compare the results. Afterthat the histopathological study done by Dr.Dhea Abdul Alhussain University of Baghdad/ Collage of veterinary occurred by several steps include tissue collection from laboratory animals, fixation of tissue sample, dehydration, clearing, wax infiltration, blocking (embedding), and Sectioning.

Results

A-Preliminary study

Montelukast after fourteen days from induction of pulmonary fibrosis by bleomycin, show the dose of 20mg/kg is the most effective and safe.

B- Main study

Montelukast affects pulmonary fibrosis as shown in the below figures

Control group

Sections of the normal lung were illustrated in Figure 1 after 28 days

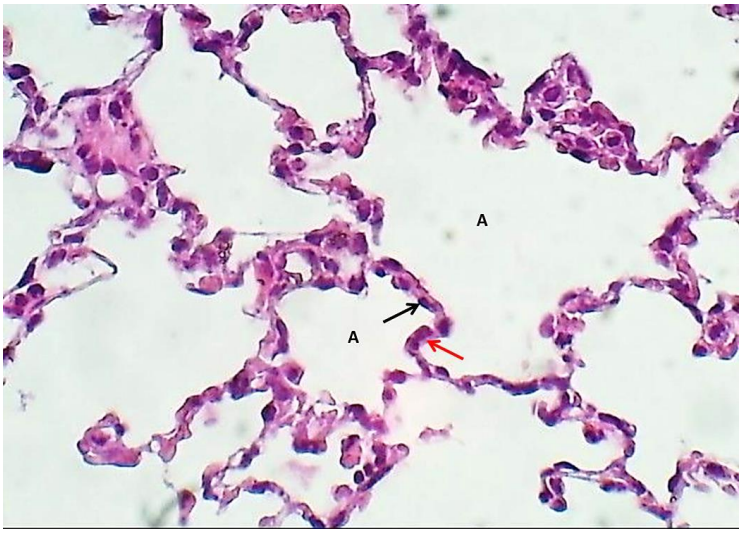

Figure (1): Section of the lung (control) shows: alveoli (A), pneumocyte type I (black arrow) & pneumocyte type - II (Red arrow). hematoxylin and eosin stain.400x.

Induction group

Sections of pulmonary parenchyma showed severe interstitial pneumonia and fibrosis characterized by massive disappearance of pulmonary parenchyma that was replaced by an inflammatory infiltrate and medial thickening of the pulmonary artery. Most alveoli revealed severe alveolar atrophy and edema, and some alveoli were filled with laminated calcified spheroid bodies. Small-sized pulmonary artery showed per vascular lymphocytic aggregation with marked dystrophy of the medial layer (figure 2).

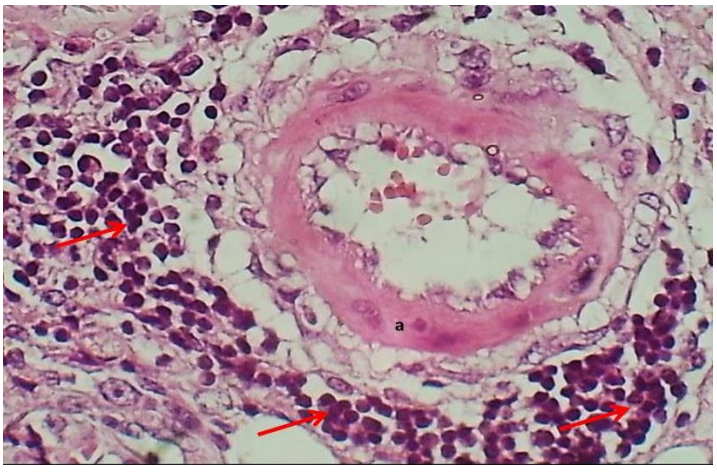

Figure (2): Section of the pulmonary artery (Induction after 28 days) shows: per vascular lymphocytic coughing (Red arrows), with marked dystrophy of medial layer (a). hematoxylin and eosin stain stain.400x.

Pirfenidone group

Most sections of pulmonary parenchyma showed normal pulmonary artery (a), alveoli (A), mild interalveolar sepat congestion, and mild bronchitis containing exudate and vascular dilation. The pulmonary interstitium showed mild interalveolar sepat vascular dilation and congestion with little lymphocytic infiltrate (figure 3).

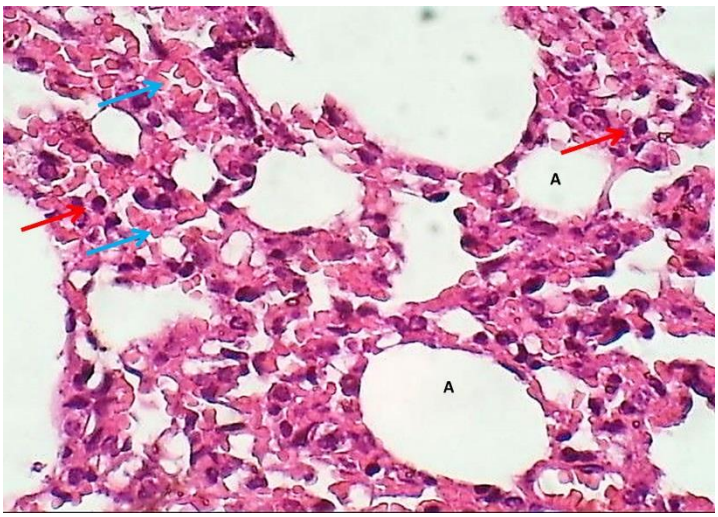

Figure (3): Section of the lung (Pirfenidone group - after 28 days) shows: normal alveoli (A), mild interalveolar sepat congestion (Blue arrows) & little lymphocytic infiltrate (Red arrows). hematoxylin and eosin stain stain.400x.

Montelukast (treatment) group

Section of pulmonary parenchyma showed normal appearance of bronchus, pulmonary vessels alveolar sac & alveoli (figure 4)

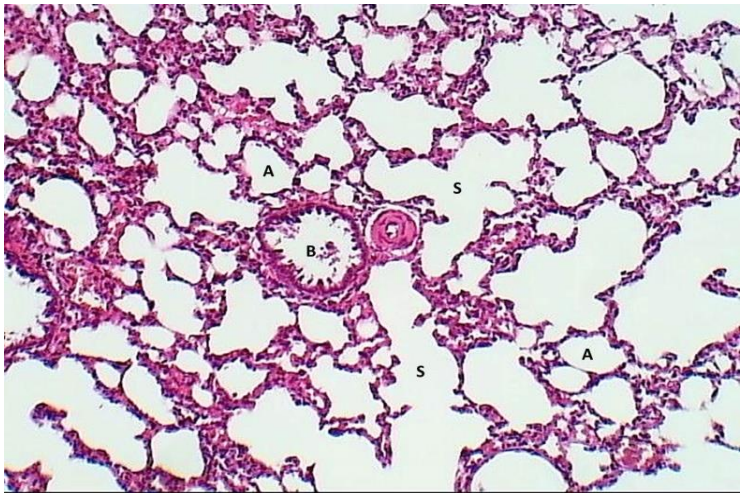

Figure (4): Section of the lung (treatment group - after 28 days) shows normal appearance of bronchioles (B), pulmonary alveolar sac (S) & alveoli (A) .H&E stain.400x.

Combination group

Section of pulmonary parenchyma showed a normal appearance of lung parenchyma involving pulmonary blood vessels, bronchiole tree, alveoli, alveolar sac & interstitial tissue (figure 5).

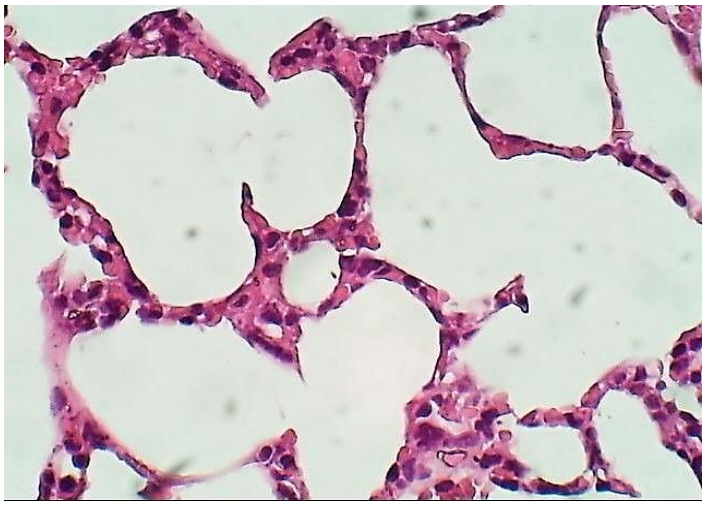

Figure (5): Section of the lung (combination group - after 28 days) shows: normal inter alveolar tissue & alveoli. hematoxylin and eosin stain stain.400x.

Discussion

According to the preliminary study, the dose of 20mg/kg is the most effective and safe because this dose shows the lung near control.

The control group shows a normal pulmonary appearance, pulmonary artery, and alveoli.

In the induction group, bleomycin lead to the proliferation of spindle cells fibroblast and type II pneumocytes within the alveoli then the infiltration of inflammatory cells after that alveoli revealed severe alveolar atrophy and edema, and some alveoli filled with laminated calcified spheroid bodies. The pulmonary interstitium revealed severe interstitial thickening associated with infiltration of macrophages, myofibroblasts, fibroblasts, and lymphocytes that lead to elevated inflammatory cytokines and cytokines that cause fibrosis as transforming growth factorβ1(TGFβ1) these results confirmed by [14], [15], and [16] where bleomycin cause injury of endothelial cells causing activation of fibroblast and accumulation of extracellular matrix finally lead to lung fibrosis.

In the pirfenidone group, pirfenidone also showed a significant decline in pulmonary fibrosis where the pulmonary artery and alveoli are normal with mild interalveolar septa congestion, and mild bronchitis contained exudate and vascular dilation this result was confirmed by [17] and [18] where administration of pirfenidone twice daily for two weeks lead to reduce the number of myofibroblasts, extracellular matrix when compared with bleomycin group.

In the montelukast group, significantly decline in pulmonary fibrosis where pulmonary parenchyma showed a normal appearance of bronchus, pulmonary vessels alveolar sac & alveoli this result came in agreement with[19], [12], and [20] where administration of montelukast causing decline of collagen content, extracellular matrix, fibroblasts, myofibroblast and back to control.

The combination therapy had an effect on lung histology and the lung architecture appeared to be almost normal.

In conclusion, montelukast after twenty-two days from starting the treatment restored the histopathological changes induced by bleomycin.

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https://uomustansiriyah.edu.iq/web_college.php?id_dept=4

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