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Research Article
Effect of Melatonin and Zafirlukast in Acute Lung Injury

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Abstract

Objective: In the present investigation, effect of melatonin and zafirleulast in acute lung injury model through NF-kappa beta (NF-κβ) mediated anti-inflammatory signaling pathway was studied. **Materials and Methods:** Acute lung injury was induced by lipopolysaccharide in mice with the animals were treated with melatonin and zafirlucast alone and combination to assess its effect on various inflammatory mediators IL-1 beta, IL-6, IL-10 and TNF-α. The effect on myeloperoxidase activity, PMN cells and the NF-κβ expressions has been evaluated in treatment groups. **Results:** In treatment with melatonin and zafirlucast showed amelioration of lung injury as it decreased level of proinflammatory mediators' increase in case of lung injury. In case of combination of treatment shows potentiation of activity as level of pro-inflammatory mediators was significantly decreased. The combination of melatonin and zafirlucast significantly reduced expression of NF-κβ as compared to treatment alone in lipopolysaccharide induced acute lung injury in mice. **Conclusion:** In conclusion, beneficial effect of melatonin and zafirlucast in acute lung injury model whereas combination of both results in potentiation of the anti-inflammatory effect in acute lung injury.

Key words: Melatonin, zafirlucast, acute lung injury, NF-κβ, TNF-α

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Acute Lung Injury (ALI) is increasing due to environmental changes such as pollens in air and industrialization which through air pollutants. Even ALI is initiation of severe respiratory inflammation due to bacterial, fungal and viral infections, bacterial pneumonia, burns and sepsis. Acute lung injury was induced through pro-inflammatory mediators and damage to surface epithelial cells results further provocation of inflammation. Pyrogens are of lipopolysaccharide in nature which results in induction of inflammation[1,2].

Melatonin, peptide from pineal gland functions as skin pigment. Melatonin is hormone having its beneficial effect from cardio metabolic disorder, various cancers through anti-inflammatory and antioxidant actions. Melatonin is reported as beneficial in an acute lung ischemia reperfusion injury. Melatonin and its reported anti-inflammatory actions in acute lung injury[3-5].

Zafirlucast is known cysteineyl leukotriene antagonist having beneficial effects in asthma and inflammation of lungs through amelioration of progressive inflammation. Zafirlucast has been wade range of pharmacological activity because of anti-inflammatory actions involved in various disease conditions[6,7]. Melatonin and zafirlucast has been reported for beneficial effect on pro-inflammatory mediators. Andrographlide, matrin and phencyclidine reported to ameliorated NF-κβ pathaway in LPS induced acute injury in mice[2,8,9]. The both agents have its effect on protein expression of NF-κβ. In the present therapies available for acute lung injuries has been limited due to getting resistance to microbes causing inflammation. Hence, more beneficial combination therapy in case of acute lung injury needs to be explored.

In nutshell, the study has been carried out to investigate the effect of melatonin and zafirlucast in this acute lung injury model. In this study, combination of both active agents potentiates amelioration of acute lung injury through its anti-inflammatory effect.

MATERIALS AND METHOD

Experimental design and drug treatment: Animals swiss mice weighing 24-30 g randomly divided in different groups provided with *ad libitum* water and normal pellet diet. Animals were kept in relative humidity and temperature 22-24°C with 12 h night and day alternate cycle. The animals were treated intratracheal with lipopolysaccharide 100 µg induced acute lung injury. The experimental design and animal treatment and procedures were approved by animal ethics committee of the institute (Protocol No. ZHM 9/15). One milliliter normal saline solution 0.9% NaCl w/v introduced in lungs with help of insertion of cannula made of polypropylene at 37°C and after 5 min recovery made. The fluid obtained from lavage was centrifuged at 500×g for 10 min at 4°C of which supernatant was separated and stored at -80°C for cytokine analysis. The cell pellet was re-suspended in 0.25 mL of saline solution and cells count was performed on automated cell counter.

Cytokines in BAL fluid: Cytokines in BAL fluid such as TNF alpha, IL-1β, IL-6 and IL-10 were measured by using ELISA method. After follow up of KIT manufacturer's instructions, the plates were automatically measured under microplate reader. The sensitivity of detector for TNF-α and IL was less than 10 pg mL^{-1}.

Western blotting: Homogenate of lung tissues of treatment group was prepared using polytron homogenizer (Fisher Scientific, USA). The protein estimation carried out using Bradford method and 20 µg of sample was separated using SDS polyacrylamide gel electrophoresis and transferred on nitrocellulose membrane. Primary antibodies were used as following: Rabbit polyclonal anti-NF-κβ (1:500) (#2762, Cell Signaling, Beverly, MA, USA), mouse monoclonal anti-β-actin (1:5000) (for internal control; A 5441, Sigma). Horseradish peroxidase-conjugated antibodies (1:500) (Amersham Pharmacia Biotech, Arlington Heights, IL, USA) were used as secondary antibodies. Band detection was performed using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech).

Statistical analysis: All data values are expressed in Mean+SEM and mean value compared with ANOVA followed by Tukey test for parametric data. A $p<0.05$ was considered significant.

RESULTS

Effect of melatonin and zafirlucast treatment on myloperoxidase activity exudate volume, PMN cells: in acute lung injury animals myloperoxidase activity was significantly increased while treatment of melatonin and zafirlecast showed significant amelioration in MPO activity ($p<0.01$) (Fig. 1) and in case of lung injury exudate volume was increased than normal animals which was significantly

reduced on treatment with melatonin and zafirlecast alone ($p<0.01$), whereas combination of melatonin and zafirlucast significantly reduced exudate than melatonin or zafirlucast alone, ($p<0.001$) (Fig. 2). Effect of melatonin and zafirlecast treatment on Polymorphonuclear cells (PMN cells) indicates significant reduction in PMN cells with melatonin, zafirlucast and in the combination (Fig. 3).

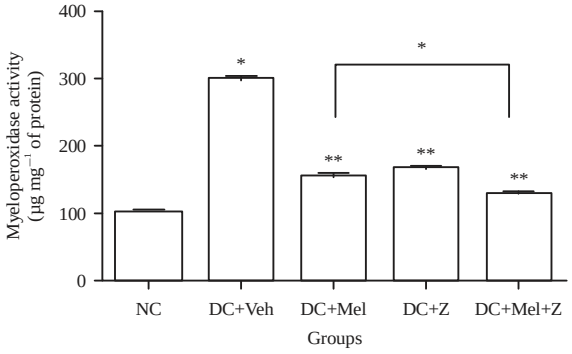

Fig. 1: Effect of melatonin and zafirlecast treatment on myloperoxidase activity, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

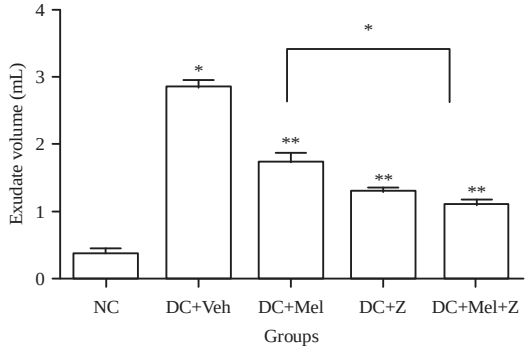

Fig. 2: Effect of melatonin and zafirlecast treatment on exudate volume, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

Effect of melatonin and zafirlucast treatment on cytokines: In acute lung injury animals cytokines was significantly increased while treatment of melatonin and zafirlecast showed significant decrease in TNF-α, IL-1β, IL-6 and IL-10 levels ($p<0.01$) and in case of melatonin and zafirlucast combination cytokines was significantly decreased than animals treated alone with melatonin or zafirlucast ($p<0.001$) (Fig. 4-7).

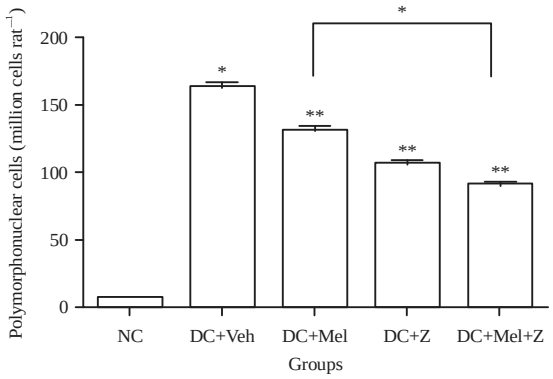

Fig. 3: Effect of melatonin and zafirlecast treatment on polymorphonuclear cells (PMN cells), NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

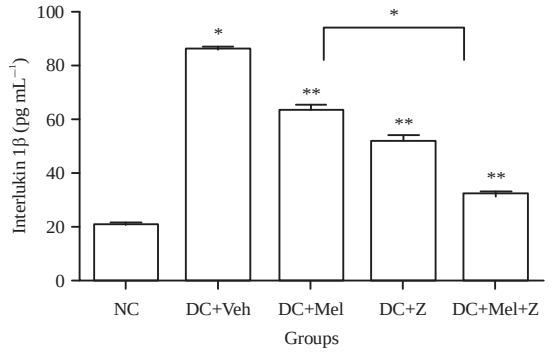

Fig. 4: Effect of melatonin and zafirlecast treatment on interlukin 1β levels, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

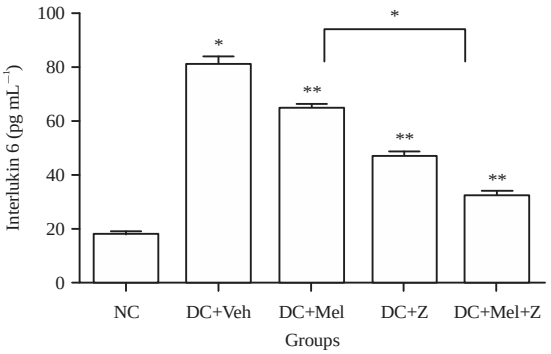

Fig. 5: Effect of melatonin and zafirlecast treatment on interlukin 6 levels, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

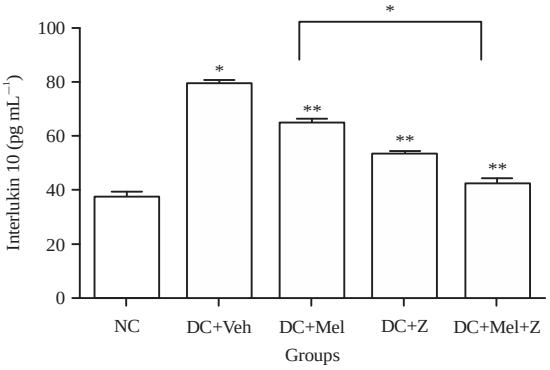

Fig. 6: Effect of melatonin and zafirlecast treatment on interlukin 10 levels, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

Effect of melatonin and zafirlucast treatment on NF-κβ protein expression: In acute lung injury, NF-κβ expression was increased significantly from normal animals while treatment with zafirlecast and melatonin showed significant amelioration of proinflammatory mediator's expression. Use of melatonin and zafirlucast significantly decreased the expression levels indicate melatonin and zafirlucast has better effect on protein expression (Fig. 8).

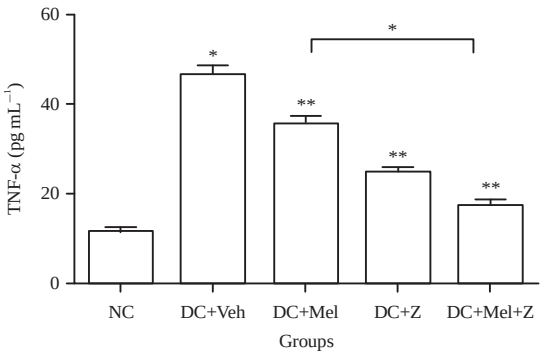

Fig. 7: Effect of melatonin and zafirlecast treatment on TNF-α levels, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

Fig. 8: Effect of melatonin and zafirlecast treatment on NF-κβ expressional levels, NC: Normal control vehicle treated, DC+Veh: Acute lung injury animals treated with vehicle, DC+Mel: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o., DC+Z: Acute lung injury animals treated with zafirlucast 10 mg kg^{-1} p.o. and DC+Mel+Z: Acute lung injury animals treated with melatonin 10 mg kg^{-1} p.o. and zafirlucast 10 mg kg^{-1} p.o.

DISCUSSION

Melatonin produces dose-dependent anti-inflammatory effect in acute, sub-acute and chronic animal model of inflammation which can be produced by anti-inflammatory drugs[10]. Melatonin exhibited protective action in bleomycin induced pulmonary inflammation and lung injury[11]. Melatonin in combination of adipose derived mesenchymal stem cell (ADMSC)-significantly reduceced acute lung ischemic reperfusion injury by inhibiting inflammation and balancing oxidative stress[12]. In radiation induced lung injury, melatonin histological analysis confirms its role as protectant in acute lung injury[13]. Melatonin on combination of steroid dexamethasone

benefited in acute lung inflammation and histological protection in carragenaninduced pleurisy in mice and supports the possible use of melatonin in combination with steroids in order to reduce the dose and the side effects related with the use of steroids for the management of inflammatory disease[14].

In lipopolysaccharide induced multiple organ damage zafirlukast showed beneficial by ameliorating oxidative stress, neutrophil infiltration and decreasing pro-inflammatory mediators[15]. Various cysteinyl leukotriene receptor antagonists inhibited bronchial hyper reactivity through IL-13 which indicates relation between cysteinyl leukotriene receptor and IL-13 in the lungs. Zafirlucast on combination with antioxidant N-acetyl-L-cysteine ameliorated endotoxin induced acute lung injury[16]. In hyperoxia induced through pulmonary changes of rat pubs protected through inhibition of leukotriene inhibition resulted in reducing hyperoxic lung injury[17,18].

In the present study, melatonin which is pituitary hormone with various beneficial pharmacological actions and zafirlucast, cysteinyl leukotriene antagonist has been evaluated for its effect on lipopolysaccharide induced acute lung injury in mice. Myeloperoxidase activity, exudate volume and polymorphonuclear cells were reduced significantly on treatment with melatonin and zafirlucast alone while combination of both showed significant amelioration than treatment alone. In this study, in lipopolysaccharide induced acute lung injury mice, melatonin and zafirlucast reduced pro-inflammatory mediators which were provoked on exposure to LPS which resulted in beginning of airway inflammation, while treatment with zafirlucast and melatonin significantly ameliorated acute lung injury in mice than the treatment alone as melatonin or zafirlucast. The NF-κβ protein expression in case of cute lung injury increased as in increased expressing on induction of inflammation while treatment of melatonin and zafirlucast resulted in decrease in NF-κβ levels which was significantly ameliorated in case of combination than melatonin and zafirlucast alone.

CONCLUSION

In conclusion, melatonin and zafilucast ameliorates LPS induced acute lung injury through decreasing pro-inflammatory mediators while combination of melatonin and zafirlucastpotentiants anti-inflammatory action of cysteinyl leukotriene antagonist action in LPS induced acute lung injury in mice.

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