Effect of a Small Natural Dietary Compound on Lung Pathology in Airway Inflammation

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Abstract

Background: Bronchodilators and corticosteroids reduce respiratory symptoms and exacerbations in diseases associated with airway inflammation but are ineffective in a subset of patients. There is a need to develop new and safe anti-inflammatories.

Aim: To investigate whether a small natural dietary compound (SNDC) can attenuate inflammation in a model of acute lung injury, when delivered directly into the lung.

Methods: SNDC was delivered in a model of acute lung injury (ALI) involving an i.n. sensitization with LPS (n=4 mice/group). Each group was subjected to a pulmonary function test (PFT) and immunohistochemistry for pro-inflammatory cytokines. It was hypothesised that the anti-inflammatory role of SNDC was mediated by dendritic cells (DC). To explore this, GMCSF induced Bone Marrow derived Dendritic Cells (BMDCs) were treated with LPS &/or SNDC and the cells analysed by flow cytometry.

Results: LPS treatment of mice resulted in increased lung resistance, and this was significantly decreased ($p<0.0001$) by co-administration with SNDC. In BMDCs, inflammatory activation markers were upregulated by LPS, and co-administering SNDC prevented this activation.

Discussion: One of the causes of airway inflammation is exposure to lipopolysaccharide (LPS-principal component of Gram negative bacteria) which activates the DCs into an inflammatory state. Our result show that airway constriction and distensibility of the lung is normalised with SNDC treatment and suggest this effect may be mediated by DC.

Conclusion: SNDCs warrant exploration as potential future anti-inflammatory compounds to treat steroid resistant inflammatory lung diseases.

INTRODUCTION

Acute Lung Injury (ALI) is characterised by airway inflammation and is very common with over four million deaths per year[1]. Airway inflammation is mainly contributed by lipopolysaccharide (LPS) which is the cell wall of gram (-)ve bacteria[2,3]. LPS which is a TLR4 agonist, is known to increase airway hyper-responsiveness (AHR)[4]. Current treatments include the use of bronchodilators and anti-inflammatory drugs such as corticosteroids (e.g. prednisone, beclomethasone, fluticasone) and cromolyn in paediatric therapy. In adults, beta-2-agonists (SABA) and leukotriene receptor agonists (LTRA) is used. However, prolonged use of these drugs has shown to cause adverse effects[5] and has led to the development of resistance[6].

Here we demonstrate the use of a small natural dietary compound in attenuating airway inflammation in a model of Acute Lung Injury (ALI) using LPS to induce inflammation.

AIMS

- To investigate whether a small natural dietary compound (SNDC) treatment is able to abrogate airway inflammation induced by lipopolysachharide (LPS).
- To study the effect of SNDC in down regulating inflammation in a model of Antigen Presenting Cells *in-vitro*.

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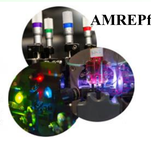


METHODS

- For Aim 1, 50 µl of 100mM SNDCs were delivered in a 24 hour model of Acute Lung Injury induced by i.n. administration of 5 µg LPS. The different groups are represented in Figure 1. Post sensitization with LPS and/or treatment, the mice were subject to a Pulmonary Function Test. The BAL fluid was collected and differential cell count was done on the cells. The mice were culled followed by isolating the lung. Two lobes from both the right and left lung were paraffin embedded. Whole lung sections (4 µm) was stained with H&E and PAS/Alcian blue followed by analysis using Olympus BX50 microscope.

To determine production of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in lung tissue, paraffin-embedded tissue was processed and immuno-stained followed by detection using DAKO Rapid EnVision immunohistochemistry (IHC) procedure. The sections were counterstained with Mayer's hematoxylin. The slides were then scanned using Leica Biosystems at 40X magnification and analysed using the Positive pixel count algorithm in Aperio Imagescope. The value of the total number of strong positive signal across the smaller airways were measured and analysed using a one-way ANOVA.

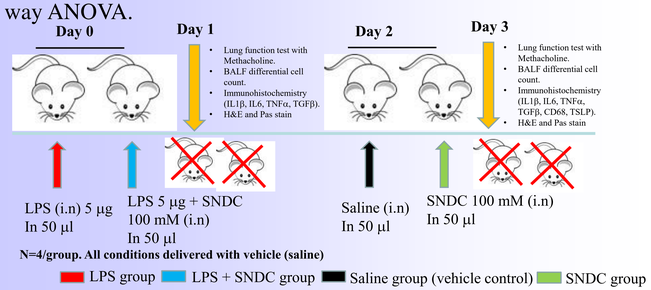


Figure 1: Study design to evaluate the anti-inflammatory effect of SNDCs

- For Aim 2, Day 6 Bone Marrow Derived Dendritic Cells were cultured using 10ng/ml GMCSF (Figure 2). The cells were then stimulated with or without LPS and SNDCs or together. After 24 hours of incubation, the cells were harvested, stained and analysed using Flow cytometry using BD Fortessa X20. Cells were gated as DCs ($CD11c^+MHCII^+$ and $GR1^-$) and the activation markers studied were CD40, CD80 and CD86.

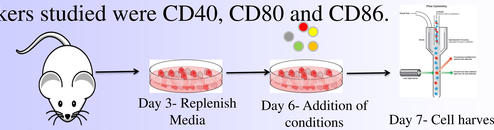
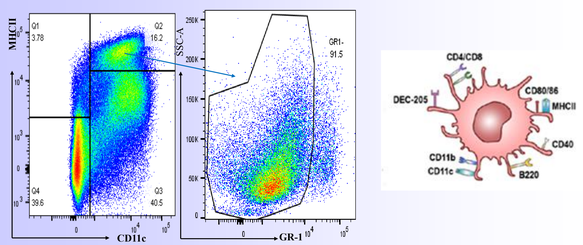
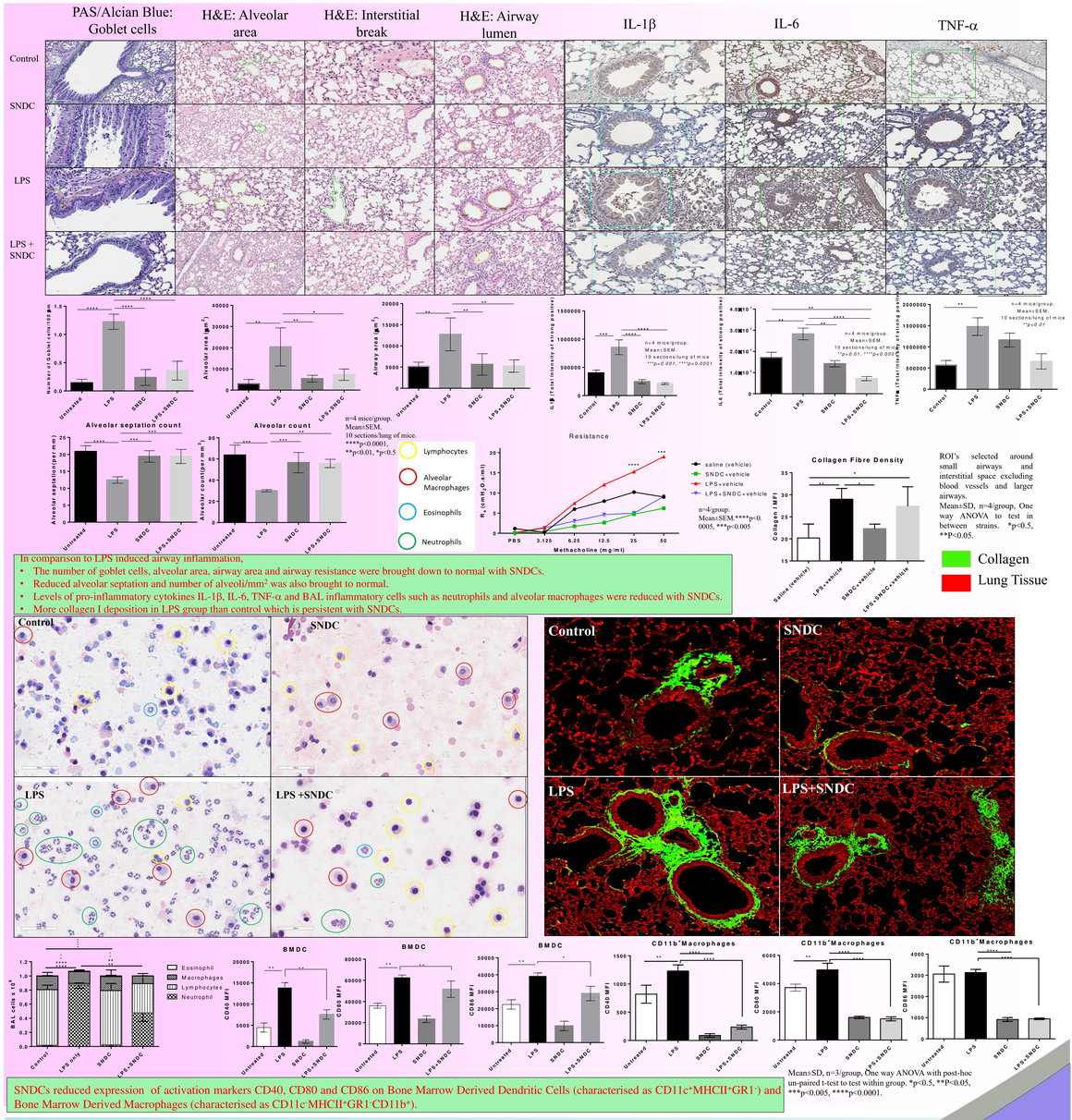


Figure 2:Culturing Bone marrow derived dendritic cells



RESULTS



In comparison to LPS induced airway inflammation,

- The number of goblet cells, alveolar area, airway area and airway resistance were brought down to normal with SNDCs.
- Reduced alveolar septation and number of alveoli/mm^2 was also brought to normal.
- Levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and BAL inflammatory cells such as neutrophils and alveolar macrophages were reduced with SNDCs.
- More collagen I deposition in LPS group than control which is persistent with SNDCs.

ROI's selected around small airways and interstitial space excluding blood vessels and larger airways. Mean±SD, n=4/group, One way ANOVA to test in between strains. *p<0.5, **p<0.05.

SNDCs reduced expression of activation markers CD40, CD80 and CD86 on Bone Marrow Derived Dendritic Cells (characterised as $CD11c^+MHCII^+GR1^-$) and Bone Marrow Derived Macrophages (characterised as $CD11c^+MHCII^+GR1^-CD11b^+$).

Mean±SD, n=3/group, One way ANOVA with post-hoc un-paired t-test to test within group. *p<0.5, **P<0.05, ***p<0.005, ****p<0.0001.

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