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REGULATION OF AIRWAY REMODELING AND OXIDATIVE STRESS IN OVALBUMIN-INDUCED ALLERGIC ASTHMA PREGNANT MOUSE

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ABSTRACT

Oxidative stress (OS) is involved in the pathogenesis of allergic asthma and airway remodeling. Among pregnant mothers, the prevalence of allergic asthma is climbing. At present, data of the imaging in branchial lumen area of allergen-induced pregnant asthma mouse model using micro-computer tomography (CT) as a modality is less reported. This study determined the effects of 100µg/200µl ovalbumin (OVA) on airway remodeling in pregnant mouse model. Eighteen (18) female BALB/c mice aged 4-5 weeks old and weighed 20-25g were divided equally into sham (G1), PBS (G2) and 100µg/200µL OVA (G3). Animals were subjected to airway sensitization (intraperitoneal injection, i.p), airway challenge (nasal instillation, n.i), and superovulation with gonadotrophins before being mated with fertile males at 1:1 ratio. At the end of the treatment, asthma was stimulated by methacholine. Animals were then euthanized on Day 10 post coitum. The serum was subjected to inflammatory biomarkers (IL5 & IL13), F2 isoprostane, total antioxidant capacity (TAC) and pregnancy hormonal (progesterone P_4 & estrogen E_2) assays. Animals were also subjected to micro-CT and hematoxylin&eosin (H&E) to determine the lumen area. Reduced bronchial lumen area ($p<0.05$) consistent with increased bronchial wall thickness ($p<0.001$) was noted in the treated group. Serum interleukin (IL)-5, IL-13, and 8-isoprostane were remarkably higher in the treated animals respectively ($p<0.001$, $p<0.05$, and $p<0.05$). In contrast, serum TAC, P4, and E2 were reduced in the treated compared to control animals ($p<0.001$, $p<0.001$, and $p<0.001$). Dose of 100 µg/200 µL OVA had induced maternal allergic airway inflammation, OS, and initiated airway remodeling in this model. The inappropriate balance of pregnancy hormone levels was indicative of possible poor implantation, placentation, fetal growth impairment, and reduced pregnancy outcome. Micro-CT modalities are preferred to be used as the result is remarkable in comparison with the H&E approach.

Keywords: allergic asthma, tocotrienol rich fraction, airway remodeling, oxidative stress, micro-CT.

INTRODUCTION

Asthma is a respiratory disorder affecting almost 13% of pregnant women worldwide (Bonham et al., 2018). In Malaysia alone, the fatality rate associated with asthma was 0.91 % of the total deaths (WHO., 2017). Allergic asthma inflammation is characterized by the recruitment of inflammatory cells, cytokines, and chemokines (Ayakannu et al., 2019). Activation of T-helper type 2 (Th2) related cytokines and interleukins (ILs) play essential roles in the exacerbation of asthma (Gurgone et al., 2020). OS on the other hand is a condition of homeostasis imbalance, characterized by raised levels of reactive oxygen species (ROS) and depleted levels of total antioxidant capacity (TAC). Appropriate sex steroid hormone levels are essential during the gestation period. Progesterone (P4) and estrogen (E2) are secreted by both ovary and placenta during pregnancy. Both hormones are reported to play significant roles in allergic diseases.

Recent study has illustrated the effects of OVA; an allergen derived from egg albumin that mimics acute asthma, on the structural changes of the airway in male mice (Paik et al., 2014). On embryonic growth, OVA-induced maternal asthma has promoted preimplantation embryonic cell death via increased cell fragmentation and zona pellucida damage (Wafriy et al., 2021). While the evidence linking asthma and pregnancy management has been highlighted (Meakin et al., 2020; Strub et al., 2016), at present, little information correlates OS as the responsible mechanism in the presentation of

airway remodeling in pregnant asthmatic animal model. The effects of allergen induced asthmatic conditions on pregnancy hormone concentrations, specifically P4 and E2 are also less described.

This study examined OVA as an allergen that induces allergic asthma, on airway remodelling in pregnant mice using micro-computed tomography (micro-CT) and hematoxylin and eosin (H&E) as the modality. The role of inflammatory-OS response and pregnancy hormones behind airway remodelling in this model was also determined.

MATERIALS AND METHOD

Eighteen (18) female BALB/c mice aged 4-5 weeks old and weighed 20-25g were divided into sham (G1), PBS (G2) and 100µg/200µL OVA (G3). Animals were subjected to airway sensitization (intraperitoneal injection, i.p), airway challenge (nasal instillation, n.i), and superovulation with gonadotrophins before being mated with fertile males at 1:1 ratio. Pregnancy was confirmed via copulatory plug, swabbing and visual inspection. At the end of the treatment, asthma was stimulated by methacholine. Animals were then euthanized on Day 10 post coitum. Following whole blood collection through cardiac puncture, the serum was subjected to inflammatory biomarkers (IL5 & IL13), F2 isoprostane, total antioxidant capacity (TAC) and pregnancy hormonal (progesterone P4 & estrogen E2) assays. Animals were also subjected to micro-CT and hematoxylin&eosin (H&E) to determine the lumen area (UiTM CARE 319/2020).

One-way analysis of variance ANOVA was applied for data associated with biochemical analysis and quantitative results of micro-CT and H&E followed by Tukey's test between all groups. Pearson correlation coefficient was used to determine the concordance between micro-CT and H&E results. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) version 25.0.0.0. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results showed reduction in the total bronchial lumen area (at 4th and 5th proximal branches and total combination) assessed under micro-computed tomography in the challenged compared to control animals (0.1255 ± 0.0612 vs 0.2513 ± 0.0832, ** = $p<0.001$). For H&E, there was a significant reduction in the bronchial lumen areas (4th and 5th proximal branches, and total combination) in 100 µg/200 µL OVA compared to PBS groups, (0.0564 ± 0.0050 vs 0.1723 ± 0.0265, $p<0.001$).

There was a raise in serum IL-13 in the treated animals (19.5162 ± 1.9595 (OVA) vs 9.7535 ± 2.8114 (PBS), * = $p<0.05$). Concurrently, serum IL-15 was also significantly increased (105.3470 ± 0.7162 (OVA) vs 41.8987 ± 2.0849 (PBS), ** = $p<0.001$). Similarly, serum 8-isoPS was notably higher (28.7533 ± 27.4463 (OVA) vs 6.8000 ± 2.4658 (PBS), * = $p<0.05$) accompanied by decreased TAC (5.5483 ± 3.6382 (OVA) vs 26.9283 ± 8.3903 (PBS), * = $p<0.001$). A decline in both serum P4 (276.6267 ± 19.7106 (OVA) vs 354.1600 ± 9.1481 (PBS), * = $p<0.001$) and E2 (117.8365 ± 0.6736 (OVA) vs 147.1697 ± 0.4549 (PBS), * = $p<0.001$) were also recorded in the treated against control groups.

In our findings, the following observations were noted: 1) the treated animals showed thicker airway walls but narrower airway lumens compared to control 2) thicker airway walls and narrower airway lumens were related to increased inflammatory-OS response and also airway remodelling. This may have subsequently led to missing daughter branches, and ultimately resulted in airway obstruction (via airway wall remodelling or airway collapse). Similarly, H&E staining showed reduced lumen area accompanied by increased bronchial wall thickness in the treated animals compared to control. Changes in the thickness might be due to the increased mucus secretion by goblet cells that eventually causes the smooth muscle and epithelial cells to become hyperplasia.

Our results suggest increased proliferation in the immune cells subtypes in the lungs of OVA treated mice as part of the inflammatory signalling pathway and call cascade initiation. This may contribute to the exhibition of allergic lung inflammation via increased IL-13 and IL-5, respectively. We postulate that the upregulated IL-5, IL-13, and 8-isoPS will subsequently affect maternal lung function and fatal growth. We suggest that 100 µg/200 µl OVA caused increased release of OS mediators resulting in compensated lumen area in our study model. Reduced serum TAC levels may also necessitate further inflammatory processes at the injury site.

We thus postulate that while normal circulating E2 and P4 may reduce maternal asthmatic conditions, decreased circulating or inappropriate E2 & P4 levels may promote asthma exacerbation, ultimately leading to various pregnancy complications and undesired pregnancy outcomes. Further studies are required to interrogate the pathophysiological processes that drive lumen narrowing over the gestational duration and their effects on the course of allergic asthma. The fluctuations of progesterone and estrogen during pregnancy, their association with maternal asthma pathogenesis, and fetal growth in the allergic airway model also warrant further elucidation.

TABLE, IMAGE AND FIGURE

Table 1. Bronchial lumen area across all groups. Data represented as mean ± SD

	RL4	RL5	LL4	LL5
PBS	0.303 ± 0.0918[a]	0.2643 ± 0.1041[a]	0.2315 ± 0.0485[a]	0.2063 ± 0.0627[a]
100 µg/200 µL OVA	0.1280 ± 0.045[b]	0.1145 ± 0.0545[b]	0.1398 ± 0.0874[b]	0.1197 ± 0.0645[b]

a,b = $p<0.001$

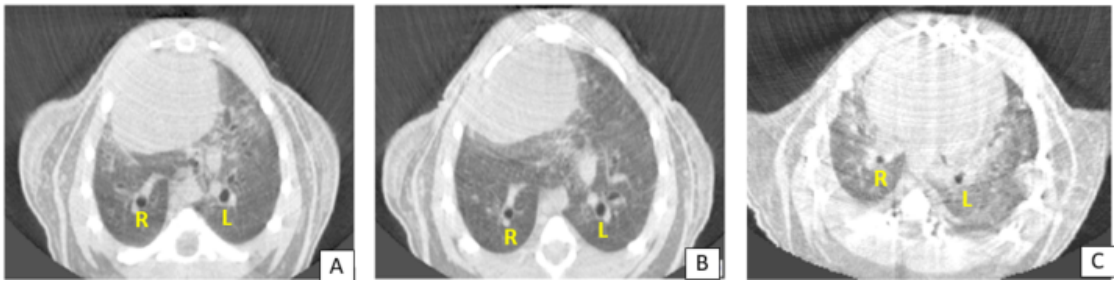
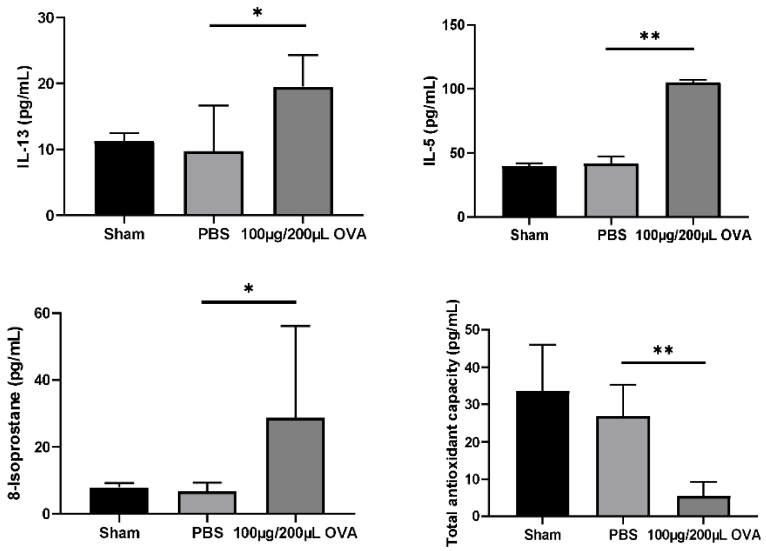


Figure 1. Micro-CT images of bronchial lumen area between A) Sham, B) PBS, and C) 100 µg/200 µL OVA groups (R = right lung; L = left lung) (300-700 BMP)



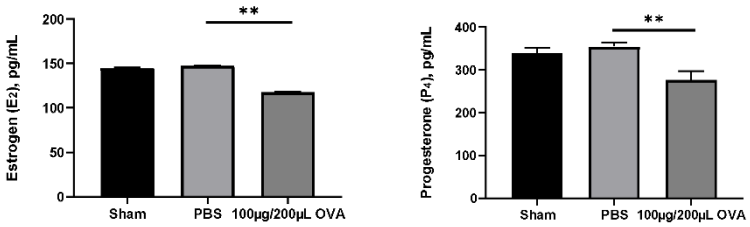


Figure 2 . Serum A) IL-13, B) IL-5, C) 8-isoPS, D) TAC, E) pregnancy hormones P_4 and F) E_2. (* = p<0.05 and ** = p<0.001).

CONCLUSION

Results demonstrated that 100 µg/200 µL OVA reduces airway lumen area and increased bronchial lumen wall thickness in pregnant allergic asthma model. This effect may be related to increased airway inflammation leading to cellular responses and airway remodeling via the OS mechanism, and imbalance in pregnancy hormone levels.

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