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House dust mite allergen causes certain features of steroid resistant asthma in high fat fed obese mice

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ABSTRACT

Obesity is a high risk factor for diseases such as cardiovascular, metabolic syndrome and asthma. Obese-asthma is another emerging phenotype in asthma which is typically refractive to steroid treatment due to its non-classical features such as non-eosinophilic cellular inflammation. The overall increased morbidity, mortality and economical burden in asthma is mainly due to steroid resistant asthma. In the present study, we used high fat diet induced obese mice which when sensitized with house dust mite (HDM) showed steroid resistant features. While the steroid, dexamethasone (DEX), treatment to high fat fed naïve mice could not reduce the airway hyperresponsiveness (AHR) induced by high fat, DEX treatment to high fat fed allergic mice could not reduce the HDM allergen induced airway remodeling features though it reduced airway inflammation. Further, these HDM induced high fat fed mice with or without DEX treatment had shown the increased activity and expression of arginase as well as the inducible nitric oxide synthase (iNOS) expression. However, DEX treatment had reduced the expressions of high iNOS and arginase I in control chow diet fed mice. Thus, we speculate that the steroid resistance seen in human obese asthmatics could be stemming from altered NO metabolism and its induced airway remodeling and with further investigations, it would encourage new treatments specific to obese-asthma phenotype.

1. Introduction

Obesity has emerged as a major risk factor for developing asthma over the decade [1]. Recent epidemiological studies suggest an association between obesity and asthma prevalence, morbidity as well as mortality. Various studies and views remark that the percentage of obese to overweight people among asthmatics could range from 30 to 50% [1]. In the light of the emerging research, obese-asthma has been classified among difficult to treat asthma sub-group due to its presentation of non-classical phenotype [2]. Akin to asthma, obese-asthma also has a heterogeneous phenotype. Obesity, in itself introduces pertinent physical changes making the disease multifactorial. For instance, abdominal and thoracic fat in obese individuals, leads to stiffening of lung movement which results in reduction of expiratory reserve volume (ERV) and functional residual capacity (FRC), creating smaller high resistance airways. However, the exact mechanism of airway hyperresponsiveness in obesity is unknown [3]. Obese-asthma is also consistently associated with lower amounts of fraction of exhaled nitric oxide (eNO), eosinophil independent inflammation, generalized inflammatory mediators such as TNF-α and IL-6, leptins and unresponsiveness towards steroids [2–7]. It has been shown that elevated body mass index (BMI) has blunted response to dexamethasone [8,9]. The cellular phenotype of obese-asthma is considered to be dominated by neutrophils and Th-17 dependent inflammation, further indicating a plausible mechanism of steroid resistance. Thus, it can be concluded that while obesity has its own repercussion on lung health, with comorbidity to asthma, it can worsen the existing symptoms of asthma. With non-Th2 cell types dominating the asthma phenotypes and its refractiveness towards steroids, there is a need of understanding the pathobiology of obese-asthma steroid resistance.

One of the potential mechanistic links between asthma and obesity is nitric oxide (NO) metabolism [10–13]. Cluster studies which show the link between late onset asthma and obesity also show an inverse association between BMI and exhaled NO (eNO) [4,5]. Reduced bioavailability of L-arginine and increased arginase activity has been associated with reduced forced expiratory volume1 (FEV1) and greater airway obstruction [6]. Hence, it could be possible that in later onset asthma, obesity could induce L-arginase methylation causing changes

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in NO metabolism leading to oxo-nitrative stress. Evidently, we have shown the reduced arginine bioavailability and increased ADMA associated oxo-nitrative stress in high fat fed obese mice [13]. In the present study, we have demonstrated that high fat diet fed obese mice which when sensitized with house dust mite (HDM) developed steroid resistant asthma like features. We further showed that these HDM-treated obese steroid resistant mice have altered NO metabolic status with an increased expression of both arginase and iNOS enzymes. So this study highlights that the steroid resistance seen in obese-asthma could be stemming from the altered NO metabolism.

2. Materials and methods

2.1. Animals and grouping

All mice experiments were as per CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines) and all experimental protocols were approved by the Institutional Animals Ethics Committee. For this study, 5–6 weeks old male C57BL/6 mice were obtained from breeding facility at CSIR-IGIB, Delhi and were acclimatized for a week to holding room environment with regular 12 h light cycle (temperature: 23° ± 2°, humidity 50–60%, Light intensity: 300 lx at floor level). There were two sets of experiments: Set A) naïve mice experiments to determine the effect of steroid, dexamethasone (DEX), on high fat diet induced airway hyperresponsiveness and Set B) house dust mite induced mice experiments to determine the effects of steroid on high fat diet induced allergic airway inflammation, airway remodeling and expressions of arginase and iNOS. In the former set, mice were divided randomly into two groups and were named as: DEX (control chow diet fed mice treated with dexamethasone), and HFA + DEX (high fat diet fed mice treated with dexamethasone). In the latter set, 36 mice were divided randomly into six groups and were named as: Naïve (control chow diet fed mice with PBS sensitization and challenge), control + HFA (High fat diet fed mice with PBS sensitization and challenge), Control + HDM (control chow diet fed mice with house dust mite allergen sensitization and challenge and vehicle treatment), HDM + DEX (control chow diet fed mice with house dust mite allergen sensitization and challenge and dexamethasone treatment), HFA + HDM (high fat diet fed mice with HDM sensitization and challenge and vehicle treatment), and HFA + HDM + DEX (high fat diet fed mice with HDM sensitization and challenge and dexamethasone treatment).

2.2. Allergen sensitization and challenge and treatment (Fig. 1)

After acclimatization to animal house and random classification, mice (both set A and B) were fed either control chow diet (Altromin 1324) or high fat diet (Research Diet Inc., D12492) according to their groups with periodical measurement of body weight. The blood glucose level was measured on day zero and on day 111 using Accucheck active^{+} glucose strip [13]. On Day 90 and 97, mice (Set B) were sensitized with either phosphate buffered saline (PBS) or 25 μg HDM (house dust mite, *Dermatophagoides pteronyssinus*, obtained from Greer Laboratories, USA or All Cure Pharma Pvt. Ltd., India)in 200 μl PBS by intraperitoneal injections. Then, these (Set B) mice were challenged with either PBS or HDM allergen (25 μg in 30 μl PBS) intranasally from Day 104 to Day 111. DEX groups (from both sets) were administered dexamethasone treatment (0.75 mg/kg weight) orally from Day 108 to Day 111.

2.3. AHR estimation

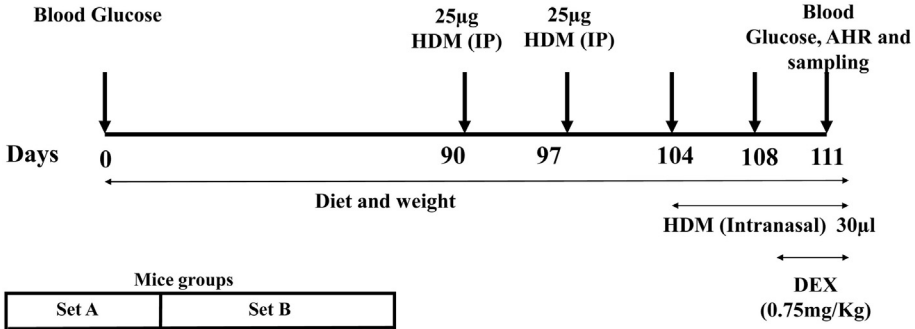
On Day 111, AHR was determined as described earlier for set A mice [16,17]. Briefly, every individual mouse that was anaesthetized was intubated and ventilated by a mechanical ventilator (Scireq, flexivent, Canada) and airway resistance was estimated with increasing concentrations of methacholine (Mch) and expressed as % baseline airway resistance assuming PBS aerosols derived values are 100%.

2.4. Lung histopathology

Set B mice were euthanized with higher dose of sodium pentothal, and lung tissues were excised and fixed in 10% buffered formalin. The fixed paraffin embedded tissue were dissected into 4 μm sections and stained with Haematoxylin and Eosin (HE), Masson Trichome (MT) and periodic acid-Schiff (PAS) staining to assess lung inflammation, collagen fiber content and goblet cell metaplasia, respectively [16].The images of stained sections were microphotographed (Nikon microscope, Model YS-100). The inflammation score was estimated by experimentally blind investigators from HE stained slides as described earlier [16]. There were separate scores for both peri-vascular (PV) and peribronchial (PB) and Total score was the sum of both PV and PB. Similarly PAS stained lung sections were used for determining PAS staining intensity [16].

2.5. Immunohistochemistry

The immunohistochemistry was performed in lung sections to determine the expressions of arginase and iNOS (Santa Cruz Biotechnology, Texas, USA) with respective secondary antibodies (Sigma, St. Louis, MO, USA or Genei, India) as described by us earlier [16]. The negative control experiments were performed using either



Mice groups

Set A	Set B
a) DEX b) HFA + DEX	a) Naive b) control + HFA c) Control + HDM d) HDM + DEX e) HFA + HDM f) HFA + HDM + DEX

Fig. 1. Experimental design: After acclimatization and random division, mice were fed either control chow diet or high fat (HFA) diet with periodical measurement of body weight. On Days 90 and 97, mice were intraperitoneally injected with PBS or 25 μg HDM and these mice were challenged with PBS or HDM allergen from Day 104 to Day 111. Some mice were treated with vehicle or dexamethasone from Day 108 to Day 111 and all mice were euthanized on day 111 after the airway hyperresponsiveness measurement.