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Research paper

Long-term allergen exposure induces adipose tissue inflammation and circulatory system injury

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ABSTRACT

The purpose of this study was to study whether allergen exposure can induce inflammation and lower the anti-inflammation levels in serum and in adipose tissues, and further develop cardiovascular injury. Our data showed that heart rate was significantly higher in the OVA-challenged mice compared to control mice. Moreover, there were higher expressions of pro-inflammation genes in the OVA-challenged mice in adipose tissues, and the expressions of anti-inflammation genes were lower. The levels of inflammation mediators were associated in serum and adipose tissues. The level of circulatory injury lactate dehydrogenase was significantly associated with the levels of E-selectin, resistin and adiponectin in the serum. The hematoxylin and eosin and immunohistochemistry stains indicated the OVA-challenged mice had higher levels of inflammation. In summary, the current study demonstrated allergen exposure can cause cardiovascular injury, and inflammatory mediators in adipose tissues play an important role in the pathogenesis of cardiovascular injury.

1. Introduction

Cardiovascular disease (CVD) constitutes a group of metabolic diseases prevalent in all countries. According to statistical data, more than 30% of the population in the USA has cardiovascular diseases [1], and CVD is third among the top ten leading causes of death. In Taiwan, about 10% of the entire population die from CVD each year, and CVD is second among the top ten leading causes of death [2]. Therefore, understanding the risk factors and mechanisms for cardiovascular disease is crucial to its prevention and the development of therapeutic strategies.

Up to now, many studies indicated the development of cardiovascular diseases is primarily due to lifestyle factors and exposure to environmental pollutants [3–9]. Inflammation plays an important role in CVD. Previous studies have indicated obesity and other factors can affect the levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in the blood, causing endothelial cell damage and produce adhesive molecules, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. Adhesive molecules can attract LDL, white blood cells and platelets into locations that result in blood vessel damage and can cause fiber caps, influencing the flow of blood and disrupting heart function [10,11]. Moreover, IL-6 and TNF-α can also produce C-reactive protein (CRP) and exacerbate the pathogenesis of CVD [12–14]. Therefore, IL-6, TNF-α, VCAM-1, ICAM-1, E-selectin, and C-reactive protein (CRP) are also important predictive markers for CVD.

People who are obese have higher amounts of subcutaneous and visceral adipose tissue, which induces changes in the levels of resistin, adiponectin, IL-6, TNF-α and interleukin-1β (IL-1β) and is related to the pathogenesis of cardiovascular diseases [15,16]. Resistin also damages endothelial cells and produces the adhesive molecules, VCAM-1, ICAM-1 and E-selectin. Therefore, many studies found higher levels of resistin in patients with CVD compared to the healthy population [15,17–22]. Adiponectin is also an important mediator of CVD. Adiponectin can reduce the risk of CVD through: (1) increases in the level of NO in blood vessels, (2) anti-oxidant activities, (3) down-regulation of endothelial cellular adhesive molecules, and (4) protection of the endothelium from apoptosis [23]. Epidemiological studies indicated patients with CVD have lower levels of adiponectin than healthy individuals [24–26], and animal studies also indicated lower levels of biomarkers with CVD in mice injected with adiponectin [27,28]. Taken together, adipokines from adipose tissue also significantly influence CVD.

We recently discovered acute exposure to allergens lowers adiponectin levels in serum and adipose tissue and concurrently induces adipose tissue inflammation [29]. This study hypothesizes allergen exposure can affect cardiovascular injury biomarkers

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through the action of adipose tissue inflammation and allergen exposure is one of the risk factors in cardiovascular injury. We tested the hypotheses by analyzing the levels and relationships of inflammatory mediators, adipokines and the biomarkers of cardiovascular injury in serum and adipose tissue and complete Hematoxylin and eosin (HE) and immunohistochemistry (IHC) staining of adipose tissue.

2. Materials and methods

2.1. Animals and animal care

Eight-week-old male C57BL/6 mice were obtained from the National Cheng-Kung University Laboratory Animal Center. The mice were grouped and kept in a constant lighting cycle (12-h light/dark), temperature, (23 ± 2 °C) and relative humidity (50–60%) with free access to food and water. All study protocols were approved by the Animal Care and Use Committee of National Cheng-Kung University.

2.2. Allergen sensitization and challenge

In the first stage, the mice were sensitized with four intraperitoneal injections of 10 μg Ovalbumin (OVA, Grade IV, Sigma-Aldrich, St Louis, MO, USA) bound to 1 mg aluminum hydroxide (Sigma-Aldrich, St. Louis, MO, USA) in 200 μl of Phosphate Buffered Saline (PBS, pH7.4) on days 1, 8, 15 and 23. The control mice were sensitized with PBS (200 μl). On day 30, in the second stage, the mice were intranasally challenged with 50 μg of OVA in 25 μl of PBS once a day, five days per week for 12 weeks. Equal volumes of PBS were used in parallel experiments as a control. Twenty-four hours after the last intranasal challenge, the mice were weighed (AE240, Mettler-Toledo, Switzerland) and sacrificed with citosol (Kyorin Pharmaceuticals, Tokyo, Japan), and the blood, bronchoalveolar lavage fluid (BALF) and adipose tissue were collected.

2.3. HR, SPB and DBP measurements

Heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were continuously taken for five days before sacrifice using a blood pressure analysis system (BP-2000 Series II, Visitech systems, United States of America) in accordance with manufacturer protocols.

2.4. Cell populations in bronchoalveolar lavage fluid (BALF)

In this study, BALF samples were drawn, and the number and population of the cells were determined to examine whether allergen exposure successfully elicits airway inflammation. Two tubes of BALF were collected in order, and each included 3 repeated, 30-s-long drawings using 1 ml of saline. The BALF was centrifuged at 4 °C and 2000 rpm for 10 min, and the pellets were re-suspended in 1 ml of RPMI 1640 medium supplemented with 10% fetal bovine serum. Twenty microliters of cell suspension were drawn for total cell counts using a hemocytometer (Marienfeld, Germany) and a microscope (BH-2, Olympus, Tokyo, Japan). The rest of the suspension was cytocentrifuged (Thermo Electron Corporation, Waltham, MA, USA) to transfer cells onto a microscopic slide, which was subsequently stained with Diff-quik (Sysmex Corporation, Tokyo, Japan). The macrophages, eosinophils, and neutrophils were differentiated by the stain, and the cell numbers in each population were counted under a microscope (CX31RBSF, Olympus, Tokyo, Japan).

2.5. Blood cardiovascular injury indexes, adiponectin, resistin, and immune markers

We measured the levels of lactate dehydrogenase (LDH), creatinine phosphokinase (CPK) and creatine kinase muscle-brain (CK-MB) with an FUJIFILM DRI-CHEM analyzer (FUJIFILM DRI-CHEM +4000i, Tokyo, Japan) using an analysis slide LDH, CK-MB and CPK (FUJIFILM, Tokyo, Japan) according to manufacturer protocols, respectively. Enzyme-linked immunosorbent assays (ELISA) were performed to determine the serum levels of adiponectin, resistin, VCAM-1, ICAM-1, E-selectin, IL-6, TNF-α (R&D System, Minneapolis, MN, USA) and total immunoglobulin E (IgE) (BD Biosciences, San Diego, CA, USA).

2.6. Inflammatory marker expression in adipose tissue

In this study, brown (interscapular) and white adipose tissues (inguinal and gonadal) were harvested and conserved in RNAlater (Protech, Taipei, Taiwan) at 4 °C for 24 h before storage at −20 °C. For RNA extraction, 1 ml of RNA Plus Extraction Reagent (Invitrogen, Carlsbad, CA, USA) was mixed with the adipose tissue, and the mixture was homogenized using a BeadBeater (BioSpec Products, Inc., Bartlesville, OK, USA). The extracted sample was further treated with an RNA pure kit (Geneaid, New Taipei, Taiwan) to increase the purity. The mRNA expressions of plasminogen activator inhibitor-1 (PAI-1), IL-6, TNF-α, IL-1β (pro-inflammatory M1 macrophage), interleukin-10 (IL-10), macrophage galactose N-acetyl-galactosamine- specific lectin 1 and 2 (Mgl1, and Mgl2) (anti-inflammatory M2 macrophage) in the adipose tissues were determined using real-time quantitative RT-PCR. Reverse transcription was performed using the SuperScript III First-Strand Synthesis Super-Mix (Invitrogen, Carlsbad, CA, USA). Quantitative real-time RT-PCR was performed with the Fast SYBR Green Master Mix (Applied Biosystems, Foster, California, USA) and the SYBR Green system (Applied Biosystems, StepOne Plus Real-Time PCR SYSTEM Thermal CyclInguinal Block, Model 4376592, Foster, CA, USA).

The data were expressed as relative gene expression to the endogenous reference, 18S rRNA, using the ΔΔCt method [30]. For comparison between the OVA- and PBS-treated groups, the level of hypoxanthine guanine phosphoribosyl transferase (HPRT) expression was used as the internal control. Primer sets were as follows: PAI-1 forward 5′-TGA TGG CTC AGA GCA ACA AG-3′ and reverse 5′-GCC AGG GTT GCA CTA AAC AT-3′; IL-6 forward 5′-GAT GCT ACC AAA CTG GAT ATA ATC-3′ and reverse 5′-GGT CCT TAG CCA CTC CTT CTG TG-3′; TNF-α forward 5′-ATG AGC ACA GAA AGC ATG-3′ and reverse 5′-TCA CAG AGC AAT GAC TCC-3′; IL-1β forward 5′-GCA GCT ATG GCA ACT GTT CCT-3′ and reverse 5′-TCA TAT GGG TCC GAC AGC ACG-3′; IL-10 forward 5′-CAG AGC CAC ATG CTC CTA GA-3′ and reverse 5′-TGT CCA GCT GGT CCT TTG TT-3′; Mgl1 forward 5′-TGA GAA AGG CTT TAA GAA CTG GG-3′ and reverse 5′-GAC CAC CTG TAG TGA TGT GGG-3′; Mgl2 forward 5′-TTA GCC AAT GTG CTT AGC TGG-3′ and reverse 5′-GGC CTC CAA TTC TTG AAA CCT-3′; and HPRT forward 5′-CTC ATG GAC TGA TTA TGG ACA GGA-3′ and reverse 5′-GCA GGT CAG CAA AGA ACT TAT AGC-3′.

2.7. Hematoxylin and eosin (HE) and immunohistochemistry (IHC) staining

In this study, the degree of inflammation in adipose tissues was determined using the HE stating method. Adipose tissues were fixed in 10% neutral formalin (Macron chemicals, Charlotte, NC, USA) for 24 h. The tissues were embedded with paraffin using a paraffin embedding mechanism (LIPSHAW) and sliced to 5 μm using a paraffin slicing mechanism (LIPSHAW). The histological

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