Prostaglandins & other Lipid Mediators 130 (2017) 16–22

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original research article

Improvement of metabolic disorders by an EP^2 receptor agonist via restoration of the subcutaneous adipose tissue in pulmonary emphysema

Takao Tsuji[a,c,*], Kazuhiro Yamaguchi[b], Ryota Kikuchi[c], Hiroyuki Nakamura[c], Ryoichi Misaka[a], Atsushi Nagai[d], Kazutetsu Aoshiba[c]

[a] Institute of Geriatrics, Tokyo Women's Medical University, 2-15-1 Shibuya, Shibuya, Tokyo 150-0002, Japan
[b] Division of Comprehensive Sleep Medicine, Tokyo Women's Medical University, 8-1,Kawada-cho, Sinjuku, Tokyo 162-8666, Japan
[c] Department of Respiratory Medicine, Tokyo Medical University Ibaraki Medical Center, 3-20-1 Chuou, Ami, Inashiki, Ibaraki 300-0395, Japan
[d] Reserch Institute for Respiratory Diseases, Shin-yurigaoka GeneralHospital, 255 Asou, Kawasaki, Kanagawa 215-0026, Japan

ARTICLE INFO

Article history:
Received 28 November 2016
Received in revised form 16 February 2017
Accepted 1 March 2017
Available online 2 March 2017

Keywords:
Subcutaneous adipose tissue
EP2 receptor agonist
Pulmonary emphysema
Metabolic disorders
COPD
Cachexia

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is often associated with co-morbidities. Metabolic disorders like hyperlipidemia and diabetes occur also in underweight COPD patients, although the mechanism is uncertain. Subcutaneous adipose tissue (SAT) plays an important role in energy homeostasis, since restricted capacity to increase fat cell number with increase in fat cell size occurring instead, is associated with lipotoxicity and metabolic disorders. The aim of this study is to show the protective role of SAT for the metabolic disorders in pulmonary emphysema of a murine model. We found ectopic fat accumulation and impaired glucose homeostasis with wasting of SAT in a murine model of elastase-induced pulmonary emphysema (EIE mice) reared on a high-fat diet. ONO-AE1-259, a selective E-prostanoid (EP) 2 receptor agonist, improved angiogenesis and subsequently adipogenesis, and finally improved ectopic fat accumulation and glucose homeostasis with restoration of the capacity for storage of surplus energy in SAT. These results suggest that metabolic disorders like hyperlipidemia and diabetes occured in underweight COPD is partially due to the less capacity for storage of surplus energy in SAT, though the precise mechanism is uncertained. Our data pave the way for the development of therapeutic interventions for metabolic disorders in emphysema patients, e.g., use of pro-angiogenic agents targeting the capacity for storage of surplus energy in the subcutaneous adipose tissue.

1. Background

Chronic obstructive pulmonary disease (COPD) is often associated with comorbidities that have a significant impact on its prognosis. Metabolic syndrome is characterized by the occurrence of a cluster of metabolic disorders, including hypertension, hyperlipidemia, diabetes mellitus, and visceral fat accumulation. Hyperlipidemia and diabetes are known to occur even in underweight COPD patients [1], and visceral fat accumulation has been reported even in COPD patients with an average BMI of 23 [2]. Low-grade chronic inflammation is considered as the mechanism underlying the development of metabolic syndrome in patients with obesity [3]. While systemic inflammation is supposed to underlie the development of metabolic syndrome in obese patients with COPD, other mechanisms may be operative in the development of metabolic disorders in patients with pulmonary emphysema who are often cachectic with wasting of the subcutaneous adipose tissue. Systemic inflammation is reported to occur in only 16% of patients with COPD. Whereas 30% of patients with COPD showed no evidence of systemic inflammation [4]. It is also suggested that there is another mechanism to explain metabolic disorders, which are found in almost one-half of the patients with COPD [5,6].

Abbreviations: COPD, chronic obstructive pulmonary disease; SAT, subcutaneous adipose tissue; EP2, Prostaglandin E2 receptor 2; Lm, mean linear intercept; NEFA, non-esterified fatty acid; HOMA-IR, Homeostatic model assessment of insulin resistance; EIE, elastase-induced pulmonary emphysema; VEGF, vascular endothelial growth factor; ELISA, enzyme-linked immunosorbent assay.

* Corresponding author at: Department of Respiratory Medicine, Institute of Geriatrics, Tokyo Women's Medical University 2-15-1 Shibuya, Shibuya-ku, Tokyo 150-0002, Japan.

E-mail addresses: tsuji.takao@twmu.ac.jp (T. Tsuji), yamaguc@sirius.ocn.ne.jp (K. Yamaguchi), reo1129@tokyo-med.ac.jp (R. Kikuchi), hiroyuki@tokyo-med.ac.jp (H. Nakamura), misaka.ryoichi@twmu.ac.jp (R. Misaka), atsushi.nagai@mt.strins.or.jp (A. Nagai), kaoshiba@tokyo-med.ac.jp (K. Aoshiba).

http://dx.doi.org/10.1016/j.prostaglandins.2017.03.001
1098-8823/

The primary function of adipocytes in the subcutaneous adipose tissue is to store fuel and provide it in times of need to non-adipose tissues. A second important function is to store any surplus energy derived from excessive food consumption, so as to protect non-adipose organs against lipid-induced trauma, namely, compartmentalization [7]. The capacity to stock surplus energy in the subcutaneous adipose tissue is known as a determinant of ectopic fat accumulation [8]. As the subcutaneous adipose tissue area has been reported to be significantly correlated with the severity of pulmonary emphysema [2,9], wasting of the subcutaneous adipose tissue is one of the clinical characteristics of patients with pulmonary emphysema. The lower capacity for storage of surplus energy due to wasting of the subcutaneous adipose tissue in patients with pulmonary emphysema may cause ectopic fat accumulation in the visceral adipose tissue, which could then lead to metabolic syndrome, including hyperlipidemia, diabetes, and visceral fat accumulation [7,8].

Angiogenesis and adipogenesis in adipose tissue show reciprocal interactions throughout life. We recently reported that both angiogenesis and adipogenesis were reduced in the subcutaneous adipose tissue in the mouse model of pulmonary emphysema induced by cigarette smoke or elastase injection [10,11]. Systemic administration of ONO-AE1-259, a Prostaglandin E2 receptor 2 (EP2) agonist with a highly selective binding affinity for the EP2 receptor and a potent stimulator of angiogenesis, stimulated angiogenesis and subsequently adipogenesis, resulting in improvement of the subcutaneous adipose tissue wasting in the mouse model of elastase-induced emphysema [11]. In this study, we demonstrated metabolic disorders such as ectopic fat accumulation and impaired glucose homeostasis in the mouse model of elastase-induced emphysema reared on a high-fat diet, and determined whether systemic administration of an EP2 receptor agonist could improve these metabolic disorders by restoration of the capacity for storage of surplus energy in the subcutaneous adipose tissue.

2. Methods

2.1. Animal treatment

All the treatments and handling of the mice at the animal facility of Tokyo Women's Medical University were approved by the Institutional Animal Care and Use Committee. Eight-week-old male C57BL/6J mice ($n = 32$) were assigned to one of three groups: saline control group ($n = 8$), saline + ONO-AE1-259 group ($n = 8$), elastase group ($n = 8$), and elastase + ONO-AE1-259 group ($n = 8$). ONO-AE1-259 binds selectively to the EP2 receptor with a high binding affinity (Ki value, 3 nM); its structure and activity are reported elsewhere [12]. The mice were anesthetized by intraperitoneal injection of sodium pentobarbital, and intratracheally instilled with 50 μL of a saline solution containing (elastase group and elastase + ONO-AE1-259 group) or not containing (saline control group and saline + ONO-AE1-259 group) 2.5 U of porcine pancreatic elastase (Sigma Chemical Co., St. Louis, MO). After an interval of 4 weeks, during which the animals were fed a standard pellet diet that provided about 5% of calories from lipids (normal diet), the mice in each group were intraperitoneally injected with 50 μL of a saline solution containing ONO-AE1-259 (100 μg/kg, Ono Pharmaceutical Co., Ltd., Osaka, Japan) (saline + ONO-AE1-259 group and elastase + ONO-AE1-259 group), or saline alone (elastase group and saline control group) on days 1–5 of each week for 4 weeks. The mice were fed a high fat diet that provided about 42% of calories from lipids (high-fat diet) during these 4 weeks. Thereafter, all the mice were fed a normal diet again for 2 weeks. Mice were allowed free access to food and water during the experimental period, while the daily food consumption was measured at 0, 3, and 6 weeks. The daily level of voluntary exercise was measured at 0 and 6 weeks by placing the mice in a separate cage equipped with turning wheels linked to a counter of turning cycles [13]. The mice were then sacrificed with a lethal dose of sodium pentobarbital at 10 weeks after the elastase/saline injection. Adipose tissue specimens from the animals (epididymal adipose tissue as visceral fat and the subcutaneous adipose tissue as subcutaneous fat) were prepared for histologic analyses. The lungs were inflated and fixed by intratracheal instillation of 10% formalin at a constant pressure of 25 cm H_2O, and embedded in paraffin, sectioned mid-sagittally (3 μm), and stained with hematoxylin and eosin for light microscopy.

2.2. Tissue histology

Morphometric analysis by light microscopy was conducted of the left lung, subcutaneous adipose tissue, and visceral adipose tissue by an investigator (TT) blinded to the group allocation of the animals. For the light-microscopic examination, mid-sagittal sections of all samples were examined at 200× magnification with an Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan). Thirty microscopic fields in each slide were randomly sampled, as described previously. To determine the mean linear intercept (Lm), the alveolar wall intersections were counted by placing an array of test grid lines diagonally on the video image, and the value of Lm was calculated using the formula Lm = 2LT/Iw, where Iw was the number of times the test line intersected the alveolar wall, and LT was the length of the test line. The adipocyte size was determined on hematoxylin & eosin-stained paraffin sections by quantifying the actual area of ≥400 discrete adipocytes per individual using Winroof image processing software (Universal Imaging).

2.3. Immunohistochemistry

Paraffin-embedded adipose tissue sections were deparaffinized in xylene, stained for blood vessels with biotin-conjugated lectin from Bandeireaea (Griffonia) Simplicifolia (1:100 dilution; BSI lectin; Sigma-Aldrich, St. Louis, MO) and fluorescein streptavidin (Vector Laboratories, Burlingame, CA). Twenty microscopic fields on each slide were randomly selected and examined with an epifluorescence microscope (Olympus BX60) at 400× magnification. Blood vessel density (the number of blood vessels per unit surface area) and adipocyte density (the number of adipocytes per unit surface area) were determined. The blood vessel to adipocyte ratio (blood vessel density relative to adipocyte density) was calculated by dividing the number of blood vessels per unit surface area by the number of adipocytes per unit surface area [13,14].

2.4. Metabolic profile

The fasting plasma levels of insulin and non-esterified fatty acid (NEFA) were determined using the commercially available Quantakine kits (R&D Systems), as per the manufacturer's instructions. Measurement of each sample was performed in duplicate. Homeostatic model assessment was applied to estimate the degree of insulin resistance (HOMA-IR): HOMA-IR = (insulin × glucose)/405 [15].

2.5. Statistical analysis

Data are expressed as means ± SEM. Differences were tested for statistical significance by analysis of variance with Scheffe's post hoc analysis. The statistical analysis was performed using StatView software for Macintosh (Abacus Concepts, Inc., Berkeley, CA).