Brain, Behavior, and Immunity xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Visceral adipose tissue inflammation is associated with age-related brain changes and ischemic brain damage in aged mice

Jin A. Shin [a,b,1], Sae Im Jeong [a,b,1], Minsuk Kim [a], Joo Chun Yoon [b,c], Hee-Sun Kim [b,d], Eun-Mi Park [a,b,*]

[a] Department of Pharmacology, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea
[b] Tissue Injury Defense Research Center, School of Medicine, Ewha Womans University, 158-710, Republic of Korea
[c] Department of Microbiology, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea
[d] Department of Molecular Medicine, School of Medicine, Ewha Womans University, Seoul 158-710, Republic of Korea

ARTICLE INFO

Article history:
Received 14 April 2015
Received in revised form 26 June 2015
Accepted 12 July 2015
Available online xxxx

Keywords:
Aging
Adipose tissue inflammation
Blood–brain barrier
Ischemic stroke
Macrophage
Microglia
Proinflammatory cytokine
Tight junction protein
Visceral fat removal

ABSTRACT

Visceral adipose tissue is accumulated with aging. An increase in visceral fat accompanied by low-grade inflammation is associated with several adult-onset diseases. However, the effects of visceral adipose tissue inflammation on the normal and ischemic brains of aged are not clearly defined. To examine the role of visceral adipose tissue inflammation, we evaluated inflammatory cytokines in the serum, visceral adipose tissue, and brain as well as blood–brain barrier (BBB) permeability in aged male mice (20 months) underwent sham or visceral fat removal surgery compared with the young mice (2.5 months). Additionally, ischemic brain injury was compared in young and aged mice with sham and visceral fat removal surgery. Interleukin (IL)-1β, IL-6, and tumor necrosis factor-α levels in examined organs were increased in aged mice compared with the young mice, and these levels were reduced in the mice with visceral fat removal. Increased BBB permeability with reduced expression of tight junction proteins in aged sham mice were also decreased in mice with visceral fat removal. After focal ischemic injury, aged mice with visceral fat removal showed a reduction in infarct volumes, BBB permeability, and levels of proinflammatory cytokines in the ischemic brain compared with sham mice, although the neurological outcomes were not significantly improved. In addition, further upregulated visceral adipose tissue inflammation in response to ischemic brain injury was attenuated in mice with visceral fat removal. These results suggest that visceral adipose tissue inflammation is associated with age-related changes in the brain and contributes to the ischemic brain damage in the aged mice. We suggest that visceral adiposity should be considered as a factor affecting brain health and ischemic brain damage in the aged population.

1. Introduction

Ischemic stroke is an age-related disease because two-thirds of strokes occur in those over the age of 65. Thus, age is a nonmodifiable risk factor for ischemic stroke (Allen and Bayraktutan, 2008). Although ischemic stroke is a leading cause of mortality and morbidity among adults, the only effective treatment is thrombolysis, which has a limited therapeutic window (Asdaghi et al., 2012). Several potential drugs were developed in pre-clinical studies, but none have been clinically proven to be effective for ischemic stroke. One likely reason is related with the use of young healthy rodents in pre-clinical stroke studies, and therefore, the Stroke Therapy Academic Industry Roundtable (STAIR) recommends using aged animals to confirm the therapeutic effects shown in young animals (Fisher et al., 2009). When using aged animals for ischemic stroke studies, age-related physiological changes that may affect ischemic injury should be taken into account.

Normal aging is associated with an increase in inflammatory mediators leading to low-grade systemic inflammation (Salvioli et al., 2006), and systemic inflammation with aging may initiate or aggravate age-related conditions, such as cardiovascular, metabolic, and neurodegenerative diseases (Huffman and Barzilai, 2009). Because several organs and many factors are involved in the proinflammatory status that occurs with aging, the mechanism of age-associated inflammation appears to be complex (Cevenini et al., 2010). Among these organs, visceral adipose tissue, which is an endocrine organ releasing chemokines and cytokines, is likely

* Corresponding author at: Department of Pharmacology, Tissue Injury Defense Research Center, School of Medicine, Ewha Womans University, 1071 Anyangcheon-ro, Yangcheon-gu, Seoul 158-710, Republic of Korea.
E-mail address: empark@ewha.ac.kr (E.-M. Park).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bbi.2015.07.008
0889-1591/

involved in the establishment of age-related systemic inflammation (Cevenini et al., 2010). Body fat is redistributed during aging from the subcutaneous compartment to the visceral adipose compartment, resulting in visceral adipose tissue accumulation (Beaufrere and Morio, 2000). With increasing adiposity, macrophage infiltration and production of proinflammatory cytokines are increased in adipose tissue. These inflammatory mediators released from visceral adipose tissue are related to low-grade systemic inflammation (Cevenini et al., 2010).

It has been reported that visceral adiposity negatively affects brain health. In human studies examining aged people, visceral fat accumulation measured by waist-to-hip ratio or imaging studies was associated with a reduction in brain volume, cortical thickness, and cognitive performance, such as verbal memory and attention (Debette et al., 2010; Isaac et al., 2011). These studies indicate that visceral adiposity induces structural and functional changes in the brain. Furthermore, brain imaging studies in individuals with or without stroke history suggest that visceral adiposity can be a risk factor for cerebrovascular diseases, because visceral fat accumulation and an increased ratio of visceral adipose tissue to total adipose tissue strongly correlate with white matter lesions and ischemic lesions (Karcher et al., 2013; Nagura et al., 2004; Yamashiro et al., 2014). In addition to being a risk factor, visceral adiposity may have an influence on brain damage following acute ischemic insult, especially in the aged brain. Because of age-related changes in the brain, such as increases in inflammatory mediators and blood–brain barrier (BBB) permeability (Norden and Godbout, 2013; Popescu et al., 2009), elderly people may be more susceptible to inflammatory mediators released from visceral adipose tissue after ischemic stroke. Moreover, one study reported that proinflammatory cytokines in visceral adipose tissue are increased by the activation of the β-adrenergic receptor via the sympathetic nervous system following acute ischemic stroke (Wang et al., 2011); therefore we hypothesize that heighted inflammation in adipose tissue in response to ischemic injury may intensify the inflammatory responses in the ischemic brain of the elderly.

In this study, to define the role of visceral adipose tissue inflammation in age-related changes in the brain and in ischemic brain damage in aged mice, inflammatory markers were examined in the circulation, visceral adipose tissue, and brain. We then compared the levels of these markers between young and aged mice. After the removal of bilateral epididymal adipose tissues, we also compared the inflammatory markers in sham mice before and after the ischemic stroke. To define the effect of ischemic brain damage on visceral adipose tissue inflammation, we examined proinflammatory cytokine production in visceral fat following an ischemic stroke.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (6 weeks old) were purchased from Orient Bio Inc. (Seongnam, Republic of Korea) and were maintained in a specific pathogen-free animal facility at Ewha Womans University School of Medicine on a 12-h light/dark cycle at 22 ± 2 °C. Standard rodent chow and tap water were provided ad libitum, except when overnight fasting was required. Experiments were performed using young adult (2.5 months old) and grossly healthy aged (18 and 20 months old) mice. For the measurement of serum testosterone, 10 mice at 2.5 months old, 8 mice at 18 months old, and 7 mice at 20 months old were used. At the age of 19 months, 116 mice were randomly divided into the sham and visceral fat removal groups (58 mice in each). Four weeks later, 3 mice in the visceral fat removal group were excluded because of severe weight loss after surgery. In each group, 22–23 mice were used for the analysis of serum cytokine levels ($n = 10$), western blotting and real-time polymerase chain reaction (PCR) ($n = 5$), immunoglobulin G (IgG) extravasation ($n = 4–5$), and immunofluorescence staining ($n = 3$) studies at baseline prior to the ischemic brain insult. Additionally, 28 mice (15 mice for serum cytokine; 5 mice for western blotting, real-time PCR, and IgG extravasation in each; 3 mice for immunofluorescence staining) at the age of 2.5 months were used for the comparison at baseline. Ischemic stroke was induced in 82 mice; 14 mice were used for IgG extravasation and for western blotting 6 h after the ischemic stroke ($n = 4–5$ in each group), and 44 mice that survived 3 days after the ischemic stroke were analyzed for measurement of infarct volume (see Section 3.4). In total, 170 of the 183 mice studied were included in the data analysis. Group sizes were determined based on our previous in vivo model of ischemic stroke experiments, and the number of animals used was minimized to reduce animal suffering. All procedures were approved by the Institutional Animal Care and Use Committee at the Medical School of Ewha Womans University and conformed to the international guidelines for the ethical use of experimental animals.

2.2. Visceral fat removal

The surgical procedures were performed in 19-month old mice as described in a previous study (Shi et al., 2013). Briefly, mice were anesthetized with isoflurane and a single abdominal midline incision was made. Bilateral epididymal adipose tissues weighing 1–1.5 g were lifted, dissected, and removed. The sham operation was performed in the same manner, but the fat was not removed. After surgery, the abdominal peritoneum was sutured with vicryl sutures and the skin was closed with sterile wound clips. The procedures were performed 4 weeks before the ischemic insult to let the mice recover from surgery and regain body weight. Fifteen mice in each group were fasted overnight (16 ± 1 h) and then blood glucose levels were measured from the tail vein using a glucometer (CareSens II®, i-Sense Inc., Wonju, Republic of Korea) before and 4 weeks after the sham or visceral fat removal surgery. Additionally, body weights of the mice were measured before and 4 weeks after the sham or visceral fat removal, and mice that lost more than 15% of their body weight were excluded. The mortality rate was 30% after visceral fat removal.

2.3. Transient middle cerebral artery occlusion (MCAO)

Procedures for transient MCAO and cerebral blood flow (CBF) monitoring were previously described (Shin et al., 2013). After 30 min MCAO, only the mice that exhibited a greater than 85% reduction in CBF during MCAO and recovered by more than 80% after 10 min of reperfusion were included. Rectal temperature was maintained at 37.0 ± 0.5 °C during surgery and recovery until the mice regained consciousness. Animals in the control group after the sham and visceral fat removal surgery did not undergo MCAO.

2.4. Behavioral tests

Neurological scores and performance in the wire suspension test and grip strength test were examined prior to and 3 days after MCAO. A 5-point graded scoring system for neurological scores and the wire suspension test was used (Shin et al., 2015). Forelimb strength was measured with the Grip strength tester bio-GS3 (BIOSEB, Virtolles cedex, France) prior to and 3 days after MCAO. When both forelimbs of the mouse were loosened by pulling the tail, the maximal force (in newtons, N) was recorded. Each mouse

Please cite this article in press as: Shin, J.A., et al. Visceral adipose tissue inflammation is associated with age-related brain changes and ischemic brain damage in aged mice. Brain Behav. Immun. (2015), http://dx.doi.org/10.1016/j.bbi.2015.07.008