Sleeve Gastrectomy Ameliorates Mrna Expression of Matrix Metalloprotease I, III, V, IL-1a and IL-6 Genes in Substantia Nigra Tissues of Obese Rats

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Abstract

Aim: To reveal metalloproteinase (MMP)-1, MMP-3, MMP-5, interleukin (IL)-1a and IL-6 gene expressions in substantia nigra region of brain in rats which undergone sleeve gastrectomy (SG) surgery.

Methods: Rats were allocated into three groups in random, which were normal rats (Group I) (n=14), obese rats (Group II) (n=14), and obese rats subjected to SG (Group III) (n=14). MMP-1, MMP-3, MMP-5, IL-1a and IL-6 gene expressions were determined by polymerase chain reaction (PCR) and Real-time polymerase chain reaction (RT-qPCR).

Results: When normal (Group I), and obese (Group II) rats were compared, a decrease in expressions of MMP-3 and IL-6 genes was observed in Group II. When obese rats (Group II) and obese rats subjected to SG (Group III) were compared, increases in the expressions of MMP-3 and IL-6 genes were observed in Group III. This phenomenon demonstrates that SG decreases obesity and consequently increases expressions of MMP-3 and IL-6 genes.

Conclusion: These data show alterations of MMP-3 and IL-6 genes in the substantia nigra tissue of obese rats, consistent with the possibility that these changes may contribute to disease molecular background.

Keywords: Obesity; Sleeve gastrectomy; Substantia nigra; Gene expressions

Introduction

Obesity and its comorbidities have become an important community health problem worldwide [1]. Recent literature revealed that adipocytes not only store fat, but are also an endocrine organ which releases a variety of mediators [2]. These mediators may manipulate, circulating lipid levels, blood pressure, and insulin resistance [2]. Common co-morbidities related with obesity, such as hypertension, hyperlipidemia, and insulin resistance are associated with increased cardiovascular and renal disease, resulting in increased morbidity and mortality [1,2]. A growing body of evidence about the function of adipocytes reveals that they play a key role in inflammatory processes [2]. Sleeve gastrectomy (SG) is a restrictive procedure that not only results in lasting weight loss but also improves insulin sensitivity and glucose tolerance before any significant weight loss has arised [3]. However, it is unclear whether SG has affects in the central nervous system.

Metalloproteinases (MMPs) are a group of Zn$^{2+}$-endopeptidases which are classified by their ability to absorb elements of the extracellular matrix [4]. MMPs are produced mainly by neurons, microglia and astrocytes [5]. Therefore, excessive expression of MMPs may result in neuronal damage. Accumulating evidence indicates that MMPs are participated in a variety of diseases such as bacterial meningitis, stroke, multiple sclerosis, and neurodegenerative diseases [6].

Regardless of the increasing data that MMPs take part in the pathology of obesity, available data on the tissular and cellular expression of the proteins is less detailed [7-9]. Here we aimed to reveal the changes in MMP-1, MMP-3, MMP-5, interleukin (IL)-1a and IL-6 gene expressions, as determined by polymerase chain reaction (PCR) and Real-time polymerase chain reaction (RT-qPCR) in substantia nigra region of brain in rats which undergone SG surgery.

Materials and Methods

Experimental design

All animal studies were carried out with the approval of the Institutional Animal Care and Use Committee. Animals were housed at constant temperature (20-22°C) and humidity (50-60%) with a 12-h light and 12-h dark cycle. They were allowed free access to water and standard rat chow. Rats were allocated into three groups in random, which were normal rats (Group I) (n=14), obese rats (Group II) (n=14), and obese rats subjected to SG (Group III) (n=14). Obese rats were fed with a high-fat diet containing 40% additional fat to the diet. One of the limitations of this study is the short follow up of 8 weeks.

Surgical (sleeve gastrectomy) procedure

After overnight fasting, rats in the sleeve group were anesthetized with an intraperitoneal injection of 300 mg/kg chloral hydrate and placed in a supine position on a surgical board with the extremities immobilized. A sinister epigastric incision was used for the operation. The length was about 1.5-2 cm in total. The incision was kept open with a blade retractor and dissociates gastric omentum to disclose gastric cardium. The gastric cavity was closed with vascular clamps, and then gastric cavity and hemostating were cut off with a cautery. A gastric tube was created with 8-0 unabsorbable suture from the distal antrum (1.5-2 mm from the pylorus) to the Hiss angle. The fundus was completely removed (70-80% of total stomach). After the gastric tube was rebuilt, the peritoneal cavity was cleaned with saline, and then closed with 6-0 silk suture.

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In the present study, based on the results of PCR and Real-Time PCR, obese rats, which may contribute to disease molecular background, alterations of MMP-3 and IL-6 genes in the substantia nigra tissue of region of brain in rats which undergone SG surgery. Our results showed interleukin (IL)-1α and IL-6 gene expressions in substantia nigra quantitatively control the expressions of genes, decrease in MMP-3 and IL-6 expressions of MMP-3 and IL-6 genes were observed secondary to obesity. When real-time PCR analysis was performed with the intention to observe MMP-3, and IL-6 gene expressions in substantia nigra (Figures 1 and 2). When groups were compared regarding genes, decreases in the expressions of MMP-3 and IL-6 genes were observed secondary to obesity. When real-time PCR analysis was performed with the intention to quantitatively control the expressions of genes, decrease in MMP-3 and IL-6 gene expressions was determined (Figure 4).

**Results**

Within the context of this study, when normal (Group I), and obese (Group II) rats were compared, a decrease in expressions of MMP-3 and IL-6 genes was observed in obese rats (Figures 1 and 2). When obese rats (Group II) and obese rats subjected to SG (Group III) were compared, an increase in the expressions of MMP-3 and IL-6 genes was observed in obese rats subjected to SG (Figure 3). This phenomenon demonstrates that SG decreases obesity and consequently increases expressions of MMP-3, and IL-6 genes.

When groups were compared regarding genes, decreases in the expressions of MMP-3 and IL-6 were observed secondary to obesity. When real-time PCR analysis was performed with the intention to quantitatively control the expressions of genes, decrease in MMP-3 and IL-6 gene expressions was determined (Figure 4).

**Discussion**

Here we aimed to reveal the changes in MMP-1, MMP-3, MMP-5, interleukin (IL)-1α and IL-6 gene expressions in substantia nigra region of brain in rats which undergone SG surgery. Our results showed alterations of MMP-3 and IL-6 genes in the substantia nigra tissue of obese rats, which may contribute to disease molecular background. In the present study, based on the results of PCR and Real-Time PCR, decrease in the expressions of MMP-3 and IL-6 genes in obese rats were resumed after SG procedure, which revealed increase in the expressions of these genes.

Obesity is defined by a substandard inflammatory status, generating its consequences on numerous organs. Earlier literatures have found that weight loss is related with a decrease in inflammation markers [e.g. C-reactive protein (CRP)], pro-inflammatory cytokines [e.g.interleukin (IL)-6, tumor necrosis factor (TNF)], Metallothioneins (MT1A and MT2A), adipokines leptin (LEP), adiponectin (ADIPOQ), the nuclear receptor, Peroxisome Proliferator Activated Receptor γ (PPARγ) and Leptin [10-15].

Weight reduction by conservative methods is difficult to achieve, and bariatric surgery is currently the most noteworthy management of significant and sustained body weight loss in morbidly obese patients [16]. Hasani et al. showed that observed a significant difference for REE between obese patients after the surgery and controls as well as between prior-and post-surgery [17]. Recent evidence suggests that SG leads to a decreased food intake not only by mechanical restriction of the stomach but also by reducing a subject’s appetite [18]. SG is supposed to modulate meal-related hormones of the gut-brain axis such as Ghrelin (signal=hunger) or peptide YY (slows gastric emptying) or GLP-1 (decreases food intake) [19].

Traurig et al., using PCR analysis, compared obese and nonobese individuals, and revealed that MMP3 mRNA levels were decreased in preadipoctyes isolated from obese individuals, and also showed a significant negative correlation between MMP3 mRNA expressions and percent body fat [20]. Adipose tissue remodeling is mainly controlled by MMPs [21,22]. The expressions of different MMPs and tissue inhibitors of MMPs (TIMPs) were examined in adipose tissue of mouse models by Chavey et al. and Maquoi et al. [21,22]. Both studies revealed statistically significant alterations in expression levels and emphasized that adipose tissue remodeling in obese individuals is modulated by these proteins. Also in various studies, MMPs also seem to be important for adipogenesis [21-24].

There are various studies both confirming and opposing our results of reduced MMP3 expression in obese subjects’ preadipoctyes/stromal vascular cells. Maquoi et al. showed that MMP3-/- mice had improved hypertrophy of adipocytes as fed with a high-fat diet; while Alexander et al. showed that MMP3-/- mice had increased differentiation of adipocytes [25,26]. On the contrary, both Maquoi et al. and Chavey et al. revealed that MMP3 expression levels were enhanced in obese mice adipose tissue [21,22]. The explanation for the mentioned divergence is uncertain. This disparity might probably be generated by variations in model systems (human vs. mouse models, or non–diet-induced vs. diet-induced obesity) or alternatively by differences in adipose tissue depots (subcutaneous vs. gonadal). Geffken et al. revealed that physically more active individuals have lower IL-6 and CRP levels and other inflammation markers [27]. Furthermore, continuous exercise may attenuate the inflammatory process, thus decreasing levels of proinflammatory cytokines. Similarly, obese individuals are identified by having elevated levels of inflammatory markers than do skinny individuals [28], Ziccardi et al. and Abad et al. showed that weight loss due to diet reduces CRP, IL-6, and TNF levels [29,30]. Dileo, Elquist et al. Figlewicz et al. and Hommel et al. showed that leptin receptors are also expressed in the substantia nigra, which would likely influence the dorsal striatum [31-34]. Li et al. showed that suggests that the up- or down-regulation of tyrosine hydroxylase mRNA expression in the VTA, VMH, and SN is mainly due to the intake of macronutrient type rather than body weight [35]. In the present study, when normal and
consequently increases expressions of MMP-3, and IL-6 genes.

One of the limitations of this study is the short follow up of 8 weeks. However, this time period is the equivalent of 6 human years [36]. While we cannot exclude the potential for weight regain following sleeve gastrectomy, other investigators have demonstrated that weight loss is maintained at a 15-week timepoint following sleeve gastrectomy in rodents [37,38]. Li et al. showed that examined tyrosine hydroxylase mRNA expression in obese mice fed a high-fat diet. After 8 week feeding of high-fat diet mice were classified as diet-induced obese and obese-resistant according to body weight gain [35]. Dunn-Meynell et al. showed that ibotenic acid injected into the VMN, substantia nigra, pars reticulata, and pars compacta destroyed intrinsic neurons and/or their local processes and decreased low-affinity 3H-mazindol binding by 13%-22% [39]. Teske et al. showed that OXA-stimulated SPA is not secondary to enhanced arousal, propensity for SPA parallels inclination to run and that orexin action on dopaminergic neurons in SN may participate in mediation of SPA and running wheel activity [40]. Another limitation of the study is a relatively high complication rate in these rats. These results were obtained after several pilot studies, refinements of technique, and development of a rigorous perioperative-care protocol as delineated in the methods. Although we still consider our results as preliminary, they warrant a larger comprehensive study, one which would include more rats at substantia nigra in order to evaluate more precisely the role of MMPs in obesity molecular background.

Conclusion
In conclusion, the authors showed alterations of MMP-3 and IL-6 genes in the substantia nigra tissue of obese rats, which may contribute to disease molecular background for the first time in the English literature. We revealed that SG decreases molecular obesity and consequently increases expressions of MMP-3, and IL-6 genes.

References


