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Dipeptidyl peptidase IV (DPP-IV) inhibition prevents fibrosis in adipose tissue of obese mice

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ABSTRACT

Background: During the development of obesity the expansion of white adipose tissue (WAT) leads to a dysregulation and an excessive remodeling of extracellular matrix (ECM), leading to fibrosis formation. These ECM changes have high impact on WAT physiology and may change obesity progression. Blocking WAT fibrosis may have beneficial effects on the efficacy of diet regimen or therapeutical approaches in obesity. Since dipeptidyl peptidase IV (DPP-IV) inhibitors prevent fibrosis in tissues, such as heart, liver and kidney, the objective of this study was to assess whether vildagliptin, a DPP-IV inhibitor, prevents fibrosis in WAT in a mouse model of obesity, and to investigate the mechanisms underlying this effect.

Methods: We evaluated the inhibitory effect of vildagliptin on fibrosis markers on WAT of high-fat diet (HFD)-induced obese mice and on 3T3-L1 cell line of mouse adipocytes treated with a fibrosis inducer, transforming growth factor beta 1 (TGFβ1).

Results: Vildagliptin prevents the increase of fibrosis markers in WAT of HFD-fed mice and reduces blood glucose, serum triglycerides, total cholesterol and leptin levels. In the *in vitro* study, the inhibition of DPP-IV with vildagliptin, neuropeptide Y (NPY) treatment and NPY Y_1 receptor activation prevents ECM deposition and fibrosis markers increase induced by TGFβ1 treatment.

Conclusions: Vildagliptin prevents fibrosis formation in adipose tissue in obese mice, at least partially through NPY and NPY Y_1 receptor activation.

General significance: This study highlights the importance of vildagliptin in the treatment of fibrosis that occur in obesity.

1. Introduction

Obesity is one of the most common health problems in developed countries and is characterized by an increase of white adipose tissue (WAT) expansion [1]. The WAT expansion ability is dependent on extracellular matrix (ECM) [2]. Moreover, ECM remodeling is a crucial event to the main WAT biological functions, such as adipogenesis and also for maintenance of tissue architecture [2]. However, in obesity it occurs excessive ECM deposition in adipose tissue, leading to fibrosis [3]. Fibrosis is characterized by deposition of ECM components, mainly collagens, which lead ultimately to organ dysfunction [4]. A major component of collagen is hydroxyproline which is often used as an indicator of collagen content [5]. Several profibrotic factors have been implicated in the development of fibrosis but the most important and potent profibrotic factor is transforming growth factor beta 1 (TGFβ1) [4]. TGFβ1 is an important player in fibrosis development and the decrease of this factor prevents fibrosis [4]. Furthermore, it was also described that subcutaneous WAT fibrosis decreases fat mass loss induced by surgery [6]. Moreover, it was demonstrated that collagen VI-null *ob/ob* mice show ameliorations in glucose and lipid metabolism [7]. Strategies that prevent or block WAT fibrosis have potential beneficial impact on the efficacy of diet regimen or therapeutical approaches in obesity.

Dipeptidyl peptidase IV (DPP-IV) is a multifunctional enzyme, which is expressed ubiquitously, including in visceral, epididymal and omental adipose tissue [8,9]. DPP-IV is a so-called “moonlighting

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protein" as it functions as a serine protease, a receptor, a costimulatory protein, and as an adhesion molecule for collagen and fibronectin [10]. DPP-IV cleaves peptides at the N-terminal region after X-proline or X-alanine, such as glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2) and gastric inhibitory polypeptide (GIP) and also neuropeptide Y (NPY) [11,12]. DPP-IV inhibitors mainly act to activate insulin secretion, inhibit glucagon secretion, improve β-cell mass, and to lower blood glucose [13]. For these reasons, several DPP-IV inhibitors are used as oral drugs for the treatment of type 2 diabetes [13].

Besides DPP-IV inhibitors action in lowering glucose, they have also been shown to have a protective role in other non-adipose tissues [14,15]. DPP-IV inhibitors were demonstrated to have a protective effect in preventing fibrosis in several organs, such as heart, liver and kidney, both *in vitro* and *in vivo* [16–18].

NPY was shown to be a DPP-IV substrate with an important role in adipose tissue physiology. NPY was observed to induce adipocyte differentiation and proliferation in 3T3-L1 cell line [19]. NPY also has an anti-lipolytic effect in adipocytes through NPY Y_1 receptor [8]. Moreover, NPY was shown to have impact in fibrosis formation in non-adipose tissues. NPY was described to reduce fibrosis in a swine model of chronic myocardial ischemia and hypercholesterolemia [20]. NPY overexpression reduces the cardiac fibrosis development in other rat model [21]. The antifibrotic effects of NPY seem to occur through NPY Y_1 receptor. Another study observed that mice lacking NPY Y_1 receptor have more myocardial fibrosis [22].

Although DPP-IV inhibition and NPY were shown to be important in fibrosis in other tissues, the role of DPP-IV inhibition and NPY is not known in the pathogenesis of adipose tissue fibrosis in obesity. The hypothesis of this study is that DPP-IV inhibitor can prevent adipose tissue fibrosis in obesity.

Thus, the aim of this study was to investigate the role of DPP-IV inhibition, by vildagliptin, in adipose tissue fibrosis in a mouse model of diet-induced obesity and using also a 3T3-L1 preadipocyte cell line.

2. Materials and methods

2.1. In vivo experiments

All experimental procedures were performed in accordance with the European Union Directive 86/609/EEC for the care and use of laboratory animals. In addition, animals were housed in a licensed animal facility (international Animal Welfare Assurance number 520.000.000.2006) and the CNC animal experimentation board approved the utilization of animals for this project. Moreover, people coordinating the animal studies have received appropriate education (FELASA course) as required by the Portuguese authorities.

2.2. Animals

Eight-week old adult male C57BL/6 mice were purchased from Charles River Laboratories and were housed under a 12 h light/dark cycle in a temperature/humidity controlled room with *ad libitum* access to water and food. Mice were randomly divided into four groups and treated for seven weeks: two groups were maintained in normal chow diet (8% fat), one group with and the other without vildagliptin treatment (30 mg/kg/day in water). The animals of the other two groups were maintained in a high-fat diet (HFD; LabDiet - Western diet for rodents) with 40% fat, also one group with and the other without vildagliptin treatment (30 mg/kg/day in water). The chow group was composed of 13 mice. The chow group with vildagliptin treatment had 10 mice. The HFD group had 14 mice. The HFD group with vildagliptin treatment was composed of 14 mice. Body weight and food consumption were measured twice a week for a total of 7 weeks.

2.3. Intraperitoneal glucose tolerance test

Intraperitoneal glucose tolerance test (ipGTT) was performed at the sixth week of HFD. The test was performed after an overnight fast (12 *h*). The next morning mice were weighted and glycemic levels were measured using the FreeStyle Precision Neo glucometer (Abbot) (Time 0). Glucose administration was performed *via* injection (2.0 g/kg, using a 20% glucose solution in saline 0.9% NaCl) into the peritoneal cavity. Glycemic levels were measured at 15, 30, 60, 90 and 120 min after glucose administration.

2.4. Tissue collection

At week 7, mice were sacrificed with a lethal dose of halothane (2-bromo-2-chloro-1, 1, 1-trifluoroethane) followed by decapitation. Liver and WAT (epididymal fat pad) were collected. The WAT was weighted and, afterwards, samples were collected, immediately frozen in dry ice and kept at − 80 °C. Blood was collected; the serum was separated by centrifugation (2000 × *g*, 15 min) and stored at − 20 °C.

2.5. Serum triglycerides and cholesterol determination

Serum triglyceride and cholesterol levels were quantified using the automatic biochemical analyzer Integra 800 (Roche).

2.6. Serum leptin and insulin quantification

Serum levels were measured for leptin and insulin with commercially available ELISA kits from EMD Millipore. All ELISA-based measurements were performed according to manufacturers' instructions.

2.7. Tissue preparation for histological processing

Mice were sacrificed with an overdose of avertin (2.5 times of 14 μL/g, 250 mg/kg, intraperitoneally). Transcardial perfusion with phosphate solution and fixation with 4% paraformaldehyde were performed. The epididymal adipose tissue and liver were collected and postfixed in 4% paraformaldehyde and cryoprotected by incubation in 25% sucrose/phosphate buffer. After that, dry tissues were embedded in paraffin, and subsequently cut into 3 μm-thick sections in a microtome.

2.8. Hematoxylin and eosin staining

For histological analysis of paraffin sections, epididymal adipose tissue and liver were stained with hematoxylin and eosin (HE). Slides were kept for 30 min at 68 °C to melt the paraffin. After 2 washes in xylene for 3 and 2 min, slides were transferred to a glass coplin jar containing 100% ethanol for 4 min and 95% for 2 min and rinsed 2 times with distilled water for 30 s. Slides were stained in hematoxylin Gill III for 5 min and bathed 2 times in distilled water for 2 and 1 min. After that, slides were stained with eosin for 1 min and dehydrated with 2 fast rinses in water, 95% and 100% of ethanol in water for 1 min and 2 times with xylene for 2 min. Glass slides were then mounted.

2.9. Adipocyte diameter quantification

Epididymal adipose tissue sections were stained with HE. The tissue was visualized using a fluorescence microscope (Axioskop 2 Plus, Zeiss, Jena, Germany) and the images were acquired with Axiovision software (release 4.7). Axiovision software was used to measure adipocyte diameter, which is represented as the average adipocyte diameter (in μm). Adipocyte diameter was measured from four groups of mice (> 100 cells/group).