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Dipeptidyl peptidase inhibition prevents diastolic dysfunction and reduces myocardial fibrosis in a Mouse model of Western diet induced obesity☆

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ABSTRACT

Objective. Consumption of a high-fat/high-fructose Western diet (WD) is linked to rising obesity and heart disease, particularly diastolic dysfunction which characterizes early obesity/metabolic cardiomyopathy. Mounting evidence supports a role for inflammation, oxidative stress and fibrosis in the pathophysiology of metabolic cardiomyopathy. Dipeptidyl peptidase-4 (DPP-4) is a circulating exopeptidase recently reported to be elevated in the plasma of patients with insulin resistance (IR), obesity and heart failure. We hypothesized that a model of WD induced obesity/metabolic cardiomyopathy would exhibit increased DPP-4 activity and cardiac fibrosis with DPP-4 inhibition preventing cardiac fibrosis and the associated diastolic dysfunction.

Materials/Methods. Four-week-old C57BL6/J mice were fed a high-fat/high-fructose WD with the DPP-4 inhibitor MK0626 for 16 weeks. Cardiac function was examined by high-resolution cine-cardiac magnetic resonance imaging (MRI). Phenotypic analysis included measurements of body and heart weight, systemic IR and DPP-4 activity. Immunohistochemistry and transmission electron microscopy (TEM) were utilized to identify underlying pathologic mechanisms.

Results. We found that chronic WD consumption caused obesity, IR, elevated plasma DPP-4 activity, heart enlargement and diastolic dysfunction. DPP-4 inhibition with MK0626

Abbreviations: WD, Western Diet; CVD, cardiovascular disease; IR, insulin resistance; MetS, metabolic syndrome; HF, heart failure; LV, left ventricle; ROS, reactive oxygen species; RAAS, renin–angiotensin–aldosterone system; GLP-1, glucagon-like peptide 1; GIP, glucose-like insulinotrophic peptide; DPP-4, dipeptidyl peptidase-4; CD, control diet; CD + MK, control diet and MK0626; WD + MK, western diet and MK0626; MRI, magnetic resonance imaging; HOMA-IR, homeostatic model of assessment of insulin resistance; RNS, reactive nitrogen species; Col 1, collagen type 1; Col 3, collagen type 3; TGF-β, transforming growth factor-β; MCP-1, monocyte chemoattractant protein-1; 3-NT, 3-nitrotyrosine; ECM, extracellular matrix; TEM, transmission electron microscopy; NO, nitric oxide.

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in WD fed mice resulted in >75% reduction in plasma DPP-4 activity, improved IR and normalized diastolic relaxation. WD consumption induced myocardial oxidant stress and fibrosis with amelioration by MK0626. TEM of hearts from WD fed mice revealed abnormal mitochondrial and perivascular ultrastructure partially corrected by MK0626.

Conclusions. This study provides evidence of a role for increased DPP-4 activity in metabolic cardiomyopathy and a potential role for DPP-4 inhibition in prevention and/or correction of oxidant stress/fibrosis and associated diastolic dysfunction.

1. Introduction

The World Health Organization estimates that more than 1.4 billion people were overweight in 2008 and current trends predict a doubling of the overweight population by 2030. Worldwide, more deaths are now linked to being overweight or obese than underweight [1,2]. The rise in obesity and insulin resistance appears to parallel increased consumption of a Western diet (WD) high in fat and fructose [3]. Accumulating experimental evidence supports a role for components of the WD in the development of heart disease that is characterized initially as delayed myocardial relaxation in diastole [4–6]. The driving forces in the pathogenesis of this diastolic dysfunction include; increased myocardial inflammation, oxidative stress and interstitial fibrosis [7–9]. Unfortunately, no evidenced based treatments exist to reduce the mortality of the diastolic dysfunction coupled to obesity and associated with these metabolic abnormalities [10–13]. Systemic inflammation is being increasingly implicated in the pathology of diastolic dysfunction particularly of over-nutrition induced metabolic cardiomyopathy [14–17]. The linkage of metabolic cardiomyopathy with inflammation has spurred research into new treatments for diastolic dysfunction of obesity and insulin resistance (IR) [18,19]. Therapies aimed primarily at improving insulin responsiveness and substrate utilization in diastolic dysfunction have yielded mixed results [20–22]. However, recent research has uncovered an important association between increased dipeptidyl peptidase-4 (DPP-4) cellular expression/plasma activity and inflammation in states of obesity, insulin resistance and diabetes [23–25].

DPP-4 is a circulating exopeptidase that cleaves and inactivates proteins at X-proline dipeptides residues. Inhibitors of DPP-4 have a role in the treatment of diabetes by preventing DPP-4 cleavage of glucagon like peptide-1 (GLP-1) and glucose-dependent insulinotrophic peptide (GIP) which stimulate insulin secretion and suppress glucagon release [26,27]. Additionally, DPP-4 is a pleiotropic enzyme with abundant expression on multiple cell types including T cells and macrophages. DPP-4 expression on these immune cells is increased in states of obesity, IR and in poorly controlled diabetes [28,29]. DPP-4 inhibition has also been shown to reduce inflammation in the setting of IR and cardiovascular disease (CVD) [25]. While emerging evidence supports a role for DPP-4 in both impaired insulin action and inflammation, few studies have looked at DPP-4 in diastolic dysfunction. Recent studies in diabetic patients found a relationship between increased DPP-4 activity and diastolic dysfunction as well as an improvement in cardiac function following DPP-4 inhibition [30,31]. We have also recently reported improvement in diastolic dysfunction in the insulin resistant Zucker obese rat model after chronic DPP-4 inhibition [32]. However, the effects of DPP-4 inhibition on left ventricular (LV) function in the metabolic cardiomyopathy of over-nutrition with a WD remain unexplored. Moreover, oxidant stress and impaired insulin action play crucial roles in cardiac tissue remodeling with interstitial fibrosis and inflammation contributing to impaired diastolic relaxation [4]. However, the potential effects of DPP-4 inhibition on fibrosis and oxidant stress in over-nutrition induced cardiomyopathy are largely unknown.

Accordingly, we posited that chronic DPP-4 inhibition in the setting of a WD would attenuate the development of insulin resistance and cardiac diastolic dysfunction by reducing oxidant stress, inflammation and associated myocardial fibrosis. To test this hypothesis, mice were fed a WD, high in fat and fructose, for 16 weeks in the presence or absence of chronic treatment with a DPP-4 inhibitor in their diet. After 16 weeks of feeding (20 weeks of age), mice were examined for weight gain, heart weight increase, IR and DPP-4 activity. After baseline characterization, we performed cine MRI assessment of heart function followed by detailed assessment for fibrosis, inflammation and oxidant stress.

2. Methods

2.1. Animals

C57BL/6 J mice were purchased from Jackson Laboratories and cared for in accordance with National Institutes of Health guidelines. All procedures were approved in advance by the Institutional for Animal Care and Use Committee of the University of Missouri. Male mice were used for this study and divided into 4 groups; control diet (CD, n = 22), control diet with DPP-4 inhibitor (CD + MK, n = 20), Western diet (WD, n = 22) and Western diet with DPP-4 inhibitor (WD + MK, n = 22). The WD consisted of a previously published diet of high fat (46%) and high carbohydrate (41.8%) with sucrose (17.5%) and high-fructose corn syrup (17.5%) (Test Diet modified 58Y1). The control diet consisted of fat (10.2%) and carbohydrate (67.4%) with dextrose (33%) and no high fructose corn syrup similar to standard rodent chow (Test Diet 58Y2). Differences in total sodium were negligible in the two diets (supplementary table 1) [8]. MK0626, a new DPP-4 inhibitor provided by Merck pharmaceuticals, was added to mouse chow so that the final concentration in chow was 33 mg MK0626•kg^{-1} chow to achieve a dose and plasma level of approximately 10 mg•kg^{-1}•day^{-1} and 100 nmol/L, respectively. This dose was based on previous developmental studies of this DPP-4 inhibitor and clinical studies on the related FDA approved DPP-4 inhibitor, sitagliptin [33].