Archives of Oral Biology 85 (2018) 148–153

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Research paper

Dipeptidyl peptidase-4 inhibitor enhances restoration of salivary glands impaired by obese-insulin resistance

Jitjiroj Ittichaicharoen[a,b,d], Nattayaporn Apaijai[b,c,d], Pongpan Tanajak[b,c,d], Piangkwan Sa-nguanmoo[b,c,d], Nipon Chattipakorn[b,c,d], Siriporn Chattipakorn[a,b,d,*]

[a] Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand
[b] Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand
[c] Cardiac Electrophysiology Unit, Department of Physiology, Faculty of Medicine, Chiang Mai University, Chiang Mai, 50200, Thailand
[d] Center of Excellence in Cardiac Electrophysiology Research, Chiang Mai University, Chiang Mai, 50200, Thailand

ARTICLE INFO

Keywords:
Insulin
Obesity
Metabolic syndrome
Salivary gland function
Mitochondria
DPP-IV inhibitor
Vildagliptin

ABSTRACT

Objective: Chronic high-fat diet consumption causes not only obese- insulin resistance, but also leads to pathological changes in salivary glands, including increased mitochondrial dysfunction, apoptosis, oxidative stress, and inflammation. Dipeptidyl peptidase-4 inhibitor (vildagliptin) is an oral anti-diabetic drug, using for treatment of type 2 diabetes. Vildagliptin has been shown to exert beneficial effects on several organs in cases of obese-insulin resistant condition. However, the effect of vildagliptin on salivary glands impaired by obese-insulin resistance has not been investigated. The hypothesis in this study is that vildagliptin confers beneficial effects on the salivary gland impaired by obese-insulin resistance via decreasing mitochondrial dysfunction, apoptosis, oxidative stress, and inflammation.

Design: Twenty-four male Wistar rats were divided into two groups. Each group was fed with either a normal (ND; n = 8) or a high fat diet (HFD; n = 16) for 16 weeks. At week 13, the HFD-fed rats were subdivided into 2 subgroups to receive either a vehicle or vildagliptin (3 mg/kg/day) for 28 days via gavage feeding. ND-fed rats were treated with the vehicle. At the end of treatment, metabolic parameters were examined, and rats were killed. Submandibular glands were removed to appraise inflammatory markers, apoptosis and mitochondrial function.

Results: Vehicle-treated HFD-fed rats developed obese-insulin resistance with an increase in oxidative stress, inflammation, apoptosis, and mitochondrial dysfunction in the salivary glands. Vildagliptin therapy reduced oxidative stress, inflammation, apoptosis and mitochondrial dysfunction in salivary gland of HFD-fed rats.

Conclusion: Vildagliptin prevented salivary gland injury occurring due to obese-insulin resistance.

1. Introduction

Long-term consumption of a high-fat diet (HFD) can lead to the development of obese-insulin resistance, metabolic syndrome (MetS), diabetes and several vital organ disorders (Apaijai, Inthachai, Lekawanvijit, Chattipakorn, & Chattipakorn, 2016; Bonomini, Rodella, & Rezzani, 2015; Pratchayasakul et al., 2011; Vollenweider, von Eckardstein, & Widmann, 2015). Previous studies have demonstrated that obese-insulin resistance is associated with systemic inflammation, resulting in internal organ injury (Furukawa et al., 2004; Hotamisligil, Shargill, & Spiegelman, 1993). In addition, it has been demonstrated that obese people and MetS patients have a tendency to low salivation (Knas et al., 2015; Matczuk et al., 2016; Muluke et al., 2016; Rodrigues et al., 2015). Several studies have shown that obese-insulin resistance causes changes in the salivary gland, including increased oxidative stress level (Zalewska et al., 2014), mitochondrial reactive oxygen species (ROS) production (Furukawa et al., 2004), and cellular apoptosis (Shikama et al., 2013). Obesity itself caused remarkable changes in salivary antioxidant/oxidant homeostasis by initiating an increase in lipid peroxidation and a decrease in anti-oxidants (Al-Rawi, 2011; Knas et al., 2015; Su et al., 2012). We have recently reported that mitochondrial dysfunction, increased inflammatory markers and apoptosis markers occurred in the salivary glands of obese-insulin resistant rats (Ittichaicharoen et al., 2016). All of those findings suggest that the obese-insulin resistant condition or pre-diabetic condition can damage the salivary glands.

* Corresponding author at: Neurophysiology Unit, Cardiac Electrophysiology Research and Training Center, Faculty of Medicine, Chiang Mai University/Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
E-mail addresses: scchattipakorn@gmail.com, siriporn.c@cmu.ac.th (S. Chattipakorn).

http://dx.doi.org/10.1016/j.archoralbio.2017.10.015
Received 13 March 2017; Received in revised form 20 September 2017; Accepted 19 October 2017

Vildagliptin is a dipeptidyl peptidase-4 (DDP-4) inhibitor, and has been used to treat type 2 diabetes. Vildagliptin has been shown to have beneficial effects in the restoration of the dysfunction of several vital organs impaired by the obese-insulin resistant condition. The dysfunction in the heart and brain included a decrease in the mitochondrial ROS production, an improvement in mitochondrial function and a reduction in inflammation and apoptosis (Apaijai et al., 2016; N. Khan, Khan, Panda, Akhtar, & Najmi, 2015; Omar et al., 2013; Pipatpiboon, Pintana, Pratchayasakul, Chattipakorn, & Chattipakorn, 2013; Ujhelyi et al., 2014; Wu et al., 2015). However, the effect of vildagliptin on damaged salivary glands under conditions of obese-insulin resistance has never been investigated. Therefore, this study aimed to determine the effect of vildagliptin on mitochondrial function, apoptosis, oxidative stress, and inflammation in the salivary glands of HFD-induced obese-insulin resistant rats. Our hypothesis is that vildagliptin confers beneficial effects on the salivary gland by reducing salivary gland mitochondrial dysfunction, apoptosis, oxidative stress, and inflammation in HFD-induced obese-insulin resistant rats.

2. Materials and methods

2.1. Animals and experimental protocols

All experimental protocols were approved by the Faculty of Medicine, Chiang Mai University Institutional Animal Care and Use Committee, in compliance with NIH guidelines. Twenty-four male Wistar rats weighting approximately 200–220 g, were obtained from the National Laboratory Animal Center, Salaya Campus, Mahidol University, Thailand. Rats were housed under standard temperature-controlled condition with a light-dark cycle of 12:12 h. Then, the rats were divided into 2 groups; normal diet group (ND, n = 8) and HFD (n = 16). In the ND group, rats received standard laboratory chow (Mouse Feed Food No.082, C.P. Company, Bangkok, Thailand) containing 19.77% energy from fat for 16 weeks. In the HFD group, rats received a high fat diet containing 59.28% energy from fat for 16 weeks (Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2012; Pipatpiboon et al., 2013; Pratchayasakul et al., 2011). At week 13, HFD-fed rats were subdivided into 2 subgroups to receive either 0.9% normal saline solution (NSS) via gavage as a control (HFV; n = 8) or vildagliptin (3 mg/kg/day per gavage: HFVil; n = 8) for 4 weeks. At week 13, ND-fed rats, rats were also given NSS via gavage for a further 4 weeks. At week 0 and 16, blood samples were drawn from each rat from tail vein for further metabolic analysis. Oral glucose tolerance tests were carried out on all rats at the end of week 16, and then animals were killed. Submandibular glands in each rat were removed for the analysis of mitochondrial function, inflammatory levels, apoptotic levels, and oxidative stress level. The experimental protocol is shown in Fig. 1.

2.2. Chemical analysis of metabolic parameters

Plasma was used to determine all metabolic parameters. A colorimetric method (ERBA diagnostic, Mannheim, Germany) was used to determine fasting plasma glucose, cholesterol and triglyceride levels. Fasting plasma high-density lipoprotein (HDL) levels were determined using a commercial colorimetric assay kit (Biovision, CA, USA). Plasma low-density lipoprotein (LDL) levels were calculated using Friedewald's equation (Martin et al., 2013). Fasting plasma insulin levels were evaluated using a commercial sandwich enzyme-linked immunosorbent assay (ELISA) kit (Millipore, MI, USA). The homeostatic model assessment (HOMA) index was calculated to evaluate the severity of peripheral insulin resistance (Pipatpiboon, Pratchayasakul, Chattipakorn, & Chattipakorn, 2012; Pratchayasakul et al., 2011). Oral glucose tolerance test (OGTT) was performed, then the computation of area under the curve (AUC) was developed to determine insulin sensitivity (Pipatpiboon et al., 2013).

2.3. Salivary mitochondrial isolation and mitochondrial function determination

Mitochondrial reactive oxygen species (ROS) production, mitochondrial membrane potential (ΔYm) and mitochondrial swelling were used as indicators of mitochondrial function as described previously (Nattayaporn Apaijai, Pintana, Chattipakorn, & Chattipakorn, 2013). Briefly, after a rat was killed, a submandibular gland was immediately removed and homogenized with 10 mL of cold isolating buffer, containing 300 mM sucrose, 5 mM N [tris(hydroxymethyl)-methyl]-2-aminoethanesulfonic sodium salt and 0.2 mM EGTA, followed by centrifugation at 800*g* for 5 min. Then, the supernatant was collected and centrifuged at 8,800*g* for 5 min. The pellet was re-suspended in a cold respiration buffer containing 50 mM sucrose, 100 mM KCl, 10 mM HEPES and 5 mM KH_2PO_4. The isolated solution was then used immediately to investigate the mitochondrial function.

To determine mitochondrial ROS production, 2-mM 2′7′-dichlorfluorescein-diacetate dye was added to the isolated salivary mitochondrial solution, and incubated at 25 °C for 20 min. The ROS production was detected at λ_{ex} 485 nm, λ_{em} 530 nm using a fluorescent microplate reader (BioTek Instruments, VT, USA). The increase in the fluorescent intensity was considered to be in relation to an increase in ROS production.

To determine mitochondrial membrane potential, 5-mM 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetra ethylbenzimidazolcarbocyanine iodide (JC-1) dye was added to the isolated salivary mitochondrial solution, and incubated at 37 °C for 30 min. The aggregated form of JC-1 dye was detected at λ_{ex} 485 nm, λ_{em} 590 nm, and the monomer form of the JC-1 dye was detected at λ_{ex} 485 nm, λ_{em} 530 nm using a fluorescent microplate reader (BioTek Instruments, VT, USA). JC-1 aggregated/monomer form was used as an indicator of mitochondrial membrane potential.

To determine mitochondrial swelling, the absorbance of the isolated mitochondrial suspension was detected at λ540 nm using a spectrophotometer. A decreased absorbance indicated mitochondrial swelling. The mitochondrial absorbance at 30 min was normalized with the absorbance at the first min. The decrease in absorbance at 30 min/absorbance at 0 min demonstrated the rate of increase of mitochondrial swelling (Apaijai et al., 2013; Sripetchwandee, Pipatpiboon, Pratchayasakul, Chattipakorn, & Chattipakorn, 2014; Yarana, Sanit, Chattipakorn, & Chattipakorn, 2012). Mitochondrial morphology was investigated by observation using transmission electron microscopy (TEM).

2.4. Determination of submandibular gland malondialdehyde (MDA) levels

Salivary gland MDA was used as an indicator of oxidative stress; MDA was measured by using HPLC-based assay (Thermo Scientific, Bangkok, Thailand). He submandibular glands were homogenized and mixed with H_3PO_4 and thiobarbituric acid (TBA) to produce TBA reactive substances (TBARS). Then the submandibular gland TBARS concentration was determined according to a standard curve (Apaijai et al., 2013).

2.5. Western blot analysis for the analysis of the inflammation, and apoptosis

Fifty to 95 μg of total proteins isolated from salivary glands were loaded into each well, mixed with a loading buffer (5% beta-mercaptoethanol, 0.05% bromophenol blue, 75 mMTris-HCl (pH 6.8), 2% SDS and 10% glycerol), and loaded onto 10% SDS-acrylamide gels. Then, the proteins were separated by electrophoresis and transferred onto nitrocellulose membranes as previously described (Pratchayasakul et al., 2011). Immunoblotting was conducted with mouse polyclonal anti-rat NFκB p65 (No. 8242; 1:1000 dilution), p-NFκB $p65^{ser536}$ (No.3031; 1:500 dilution), pro-Caspase 3 (No. 9662), cleaved Caspase