JOURNAL OF FUNCTIONAL FOODS 7 (2014) 517–526

Available at www.sciencedirect.com

ScienceDirect

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

Fucosylated chondroitin sulphate from sea cucumber inhibits high-fat-sucrose diet-induced apoptosis in mouse pancreatic islets via down-regulating mitochondrial signaling pathway

Shiwei Hu, Jingfeng Wang, Hui Xu, Yuming Wang, Zhojie Li, Changhu Xue**

College of Food Science and Engineering, Ocean University of China, Qingdao 266003, China

ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form
25 December 2013
Accepted 3 January 2014
Available online 21 February 2014

Keywords:
Cucumaria frondosa
Fucosylated chondroitin sulphate
Pancreatic islets
Apoptosis
Mitochondrial pathway
Cytochrome *c*

ABSTRACT

Hyperglycaemia can induce pancreatic islets apoptosis. We previously found that fucosylated chondroitin sulphate from *Cucumaria frondosa* (*Cf*-CHS) exhibited anti-hyperglycaemic effects; however, its effects on pancreatic islets are lacking. This study investigated the effects of *Cf*-CHS on inhibition pancreatic islets apoptosis in high-fat high-sucrose diet (HFSD)-induced insulin resistant mice for 19 weeks. Results showed that *Cf*-CHS significantly repaired HFSD-injured pancreatic islets, decreased blood glucose, insulin, TNF-α levels, and increased adiponectin level. *Cf*-CHS significantly reduced Bid, Bax, cytochrome c, caspase 9, and caspase 3 mRNA expressions, and increased Bcl-2 and Bcl-xL mRNA expressions. *Cf*-CHS also caused significant down-regulation of t-Bid, Bax, cytochrome c in cytoplasm, caspase 9, and cleaved-caspase 3 proteins, and up-regulation of Bcl-2 and Bcl-xL proteins. Furthermore, *Cf*-CHS enhanced the effects of rosiglitazone (RSG). These indicate that *Cf*-CHS inhibits pancreatic islets apoptosis *via* inhibition mitochondrial pathway. These findings may provide a dietary intervention hyperglycaemia-induced pancreatic islets apoptosis.

1. Introduction

Type 2 diabetes mellitus is characterized by reduced insulin sensitivity attributed to insulin resistance and pancreatic β-cell dysfunction (Muoio, & Newgard, 2008; Rhodes, 2005). The inability of pancreatic β-cell to provide the body with an insufficient insulin supply to compensate for insulin resistance is an early defect in the natural history of type 2 diabetes (Moon et al., 2013). Although the functional defect in insulin secretion toward the overall morbidity remains unclear, a reduction of β-cell mass in the pancreas is a pathological hallmark in the development of type 2 diabetes (El-Azab, Attia, & El-Mowafy, 2011; Lee et al., 2011). Autopsies of human pancreatic tissue showed an approximately 60% reduction in

* *Corresponding authors*. Tel.: +86 0532 82031948.
E-mail addresses: jfwang@ouc.edu.cn (J. Wang), xuech@ouc.edu.cn (C. Xue).

Abbreviations: ANOVA, one-way analysis of variance; *Cf*-CHS, fucosylated chondroitin sulphate from *Cucumaria frondosa*; ECL, super-enhanced chemiluminescence; HE, hematoxylin and eosin; HFSD, high-fat high-sucrose diet; HOMA-IR, homeostasis model assessment of insulin resistance index; LFSD, low-fat low-sucrose diet; M-MLV, moloney murine leukemia virus reverse transcriptase; PVDF, polyvinylidene fluoride; RSG, rosiglitazone; RT-PCR, reverse transcriptase-polymerase chain reaction; SD, standard deviation

http://dx.doi.org/10.1016/j.jff.2014.01.004
1756-4646/

β-cell mass in type 2 diabetic patients relative to nondiabetic control subjects, and a 10-fold or 3-fold increase in β-cell apoptosis was noted in type 2 diabetic patients who were lean or obese, respectively (Butler, Janson, Bonner-Weir, Ritzel, Rizza, & Butler, 2003). In many multiple pharmacological therapies for type 2 diabetes, few of these therapies were reported to prevent the progressive decline in β-cell function or inhibition of its apoptosis (Hou et al., 2013). Therefore, therapeutic agents that can halt or prevent pancreatic β-cell apoptosis failure will likely have a major impact on disease progression.

Dietary-induced inflammation plays a crucial role in the development of insulin resistance and type 2 diabetes (Kang, Tsuyoshi, Han, Kawada, Kim, & Yu, 2010; Xu et al., 2003). The involvement of inflammatory mediators, including tumor mecrosis factor-α (TNF-α), in the pathogenesis of the increased β-cell apoptosis has been widely documented in human and experimental models of diabetes (Bouzakri et al., 2011; Cai et al., 2011; Uno et al., 2007). Recently, a specific role has been proposed for TNF-α in impairing insulin secretion and inducing β-cell apoptosis in pancreatic cell lines (Zhang, Dign, Dai, Wang, & Li, 2011). Adiponectin is an anti-inflammatory and insulin-sensitizing hormone that is negatively associated with insulin resistance (Blesso, Andersen, Barona, Volk, Volek, & Fernandez, 2013). It is thought that adiponectin plays a key role in down-regulation of hyperglycaemia and reduction in pancreatic islet apoptosis (Chai, Wang, Zhou, Liu, Geng, & Liu, 2011; Wijesekara, Krishnamurthy, Bhattacharjee, Suhail, Sweeney, & Wheeler, 2010). A recent study showed that adiponectin prevented pancreatic islet ischemia–reperfusion injury *via* suppression of TNF-α production (Du, He, Jiang, Wei, & Hu, 2013). Therefore, TNF-α and adiponectin play pivotal roles in the development of β-cell apoptosis.

Sea cucumber is a popular traditional marine food in China, and exhibits many biological activities because of its bioactive compounds, such as sulphated polysaccharides, saponins, and lipids (Hu et al., 2013a; Wu et al., 2013; Zhao et al., 2012; Zhong, Khan, & Shahidi, 2007). Fucosylated chondroitin sulphate (SC-CHS) is one component of the polysaccharides in sea cucumber body wall (Chen et al., 2013; Luo et al., 2013). The specific sulphation pattern, fucose branches, is particularly important for the bioactivities of SC-CHS and different from mammalian chondroitin sulphate (Glauser, Pereira, Monteiro, & Mourao, 2008). Anti-coagulation (Chen, Xue, Yin, Tang, Yu, & Chai, 2011; Chen et al., 2012; Glauser et al., 2008; Mourao, Boisson-Vidal, Tapon-Bretaudiere, Drouet, Bros, & Fischer, 2001), anti-thrombosis (Chen et al., 2012, 2013), and anti-tumor (Borsig, Wang, Cavalcante, Cardilo-Reis, Ferreira, & Mourao, 2007) bioactivities of SC-CHS have been reported in several papers. Our previous studies have shown that SC-CHS could reduce blood glucose via promotion of hepatic glycogen synthesis and skeletal muscle tissue glucose uptake in insulin resistant mice (Hu, Chang, Wang, Xue, Li, & Wang, 2013; Hu et al., 2013). However, its effect on pancreatic islets has not been understood. In the present study, we aimed at evaluating the effects of SC-CHS isolated form *Cucumaria frondosa* (*Cf*-CHS) on inhibition of pancreatic islets apoptosis in insulin resistant mice and determining the mechanisms involved in the apoptosis-related gene expression in islets.

2. Materials and methods

2.1. Materials

Dried *C. frondosa* was purchased at a seafood market in Qingdao, China. It was identified by Professor Yulin Liao of the Chinese Academy of Sciences Institute of Oceanography (Qingdao, China). Insulin and TNF-α ELISA assays kits, TRIzol reagent were Invitrogen products (Carlsbad, CA, USA). RNase free water, dNTPs, moloney murine leukemia virus reverse transcriptase (M-MLV), random primer, and PageRuler prestained protein ladder were from TaKaRa Bio Inc (Otsu, Shiga, Japan). Rabbit anti-rat t-Bid, Bax, cytochrome c, Bcl-2, Bcl-xL, caspase 3, cleaved-caspase 3, caspase 9, β-actin polyclonal antibodies, and goat-anti rabbit antibody IgG-HRP were Cell Signaling products (Beverly, MA, USA). Glucose test kit was from Biosino Bio-Tec (Beijing, China). Western blot IP lysis buffer, BCA protein concentration kit, and super-enhanced chemiluminescence (ECL) detection kit were Applygen Technologies Inc products (Beijing, China). The primers of Bid, Bax, Bak, Bcl-2, Bcl-xL, cytochrome c, caspase 9, caspase 3, and β-actin were synthesized by ShanGon Ltd. Co. (Shanghai, China).

2.2. Preparation of *Cf*-CHS

Cf-CHS was prepared as reported previously (Hu, Chang, Wang, Xue, Li, & Wang, 2013; Hu et al., 2013). The yield was about 4.05%. The chemical composition included mainly glucuronic acid, galactosamine, fucose in mole ratio of 1:1.50:1.16. Its sulphate content was 30.07% and its molecular weight was 14.76 kDa.

2.3. Animals and experimental design

Male C57BL/6J mice, 4–5 weeks, were purchased from Vital River Laboratory Animal Center (Beijing, China; Licensed ID: SCXK2009-0007). They were housed in a 12–12 h light–dark condition at 23 ± 1 °C daily. The use of animals in this study was approved by the Ethical Committee of Experimental Animal Care at Ocean University of China. The insulin resistant model mice were established by fed a high-fat high-sucrose diet (HFSD, Research Diets, New Brunswick, NJ, USA; #D12331), which consisted 20% protein, 25% fat and 20% carbohydrates as described by Hu et al. (2013a). The mice were randomized into 6 groups of 10 animals each: control, HFSD-fed (HFSD), 1 mg/kg/d RSG-treated (RSG), 80 mg/kg/d *Cf*-CHS-treated (*Cf*-CHS), 20 mg/kg/d *Cf*-CHS plus 1 mg RSG/kg/d RSG-treated (20 *Cf*-CHS + RSG), and 80 mg/kg/d *Cf*-CHS plus 1 mg RSG/kg/d RSG-treated (80 *Cf*-CHS + RSG) groups. Mice in the control group were fed with a low-fat low-sucrose diet (LFSD, #D12328) and the others were fed with HFSD. After 19 weeks of treatment, the 8-h fasting animals were sacrificed. The blood was collected to test fasting blood glucose, serum insulin, TNF-α, and adiponectin levels. The tail of pancreas was cut off carefully to observe the histological structure of islands of langerhans and to detect apoptosis-related genes expression.