Chemico-Biological Interactions 247 (2016) 49–54

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Phycocyanin ameliorates alloxan-induced diabetes mellitus in mice: Involved in insulin signaling pathway and GK expression

Yu Ou*, Zhiheng Ren, Jianhui Wang, Xuegan Yang

School of Life Science and Technology, China Pharmaceutical University, Nanjing, China

ARTICLE INFO

Article history:
Received 11 November 2015
Received in revised form
14 January 2016
Accepted 22 January 2016
Available online 28 January 2016

Keywords:
Phycocyanin
Alloxan
Diabetes
Insulin signaling pathway
Glucokinase
Glucokinase regulatory protein

ABSTRACT

The therapeutic potential and molecular mechanism of phycocyanin from Spirulina on alloxan-induced diabetes mice was investigated. In the experiment, 4-week treatment of phycocyanin at the dose of 100 and 200 mg/kg body weight in alloxan-induced diabetes mice resulted in improved metrics in comparison with alloxan-induced diabetes group. These metrics include blood glucose levels, glycosylated serum protein (GSP), glycosylated hemoglobin (GHb) and fasting serum insulin (FINS) levels. As its molecular mode of action, phycocyanin leads to the increase of IRS-1 tyrosine phosphorylation and the decrease of IRS-1 serine phosphorylation, also accompany with increased level of Akt phosphorylation on Ser473 in the liver and pancreas in diabetic mice. In addition, phycocyanin treatment enhanced the glucokinase (GK) level in the liver and pancreas, and the glucokinase regulatory protein (GKRP) level in the liver in diabetic mice. The results suggest that phycocyanin ameliorates alloxan-induced diabetes mellitus in mice by activating insulin signaling pathway and GK expression in pancreas and liver in diabetic mice.

1. Introduction

Phycocyanin (PC) is a blue photosynthetic pigment accounting for around 15% of the total dry weight of *Spirulina platensis* and has been used as a food colorant for chewing gum, ice sherbets, soft drinks, candies and cosmetics including lipstick and eyeliners. It is also used as biochemical tracers in immunoassays due to its fluorescent properties [1]. Its therapeutic properties, including antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, anti-cancer and anti-diabetes activities have been proven [2–7].

Our previous study suggests that phycocyanin has therapeutic effect on diabetes KKAy mice [7]. Furthermore, phycocyanin administration to mice prior to alloxan injection protected them against diabetic development [6]. In the present study, we would like to investigate whether phycocyanin could ameliorate alloxan-induced diabetes mellitus in mice and its molecular mechanism.

Cells require insulin to absorb glucose from blood for use as fuel orstorage. But the glucose metabolism is altered in the patients of diabetes mellitus, due to either low levels of insulin secretion (type 1 diabetes) or abnormal resistance to insulin's effects (type 2 diabetes) [8]. When insulin binds to insulin receptor (IR), the intrinsic tyrosine kinase activity of IR is activated and autophosphorylates several tyrosine residues. Then, the activated IR phosphorylates IR substrate-1 (IRS-1) at key tyrosine residues and in turn triggers signaling transduction by activating downstream targets such as phosphatidylinositol-3 kinase (PI3K) and Akt [9]. Glucokinase (GK), also called hexokinase IV, is the rate-limiting enzyme which catalyses glucose phosphorylation in pancreatic β-cells and hepatocytes, and plays a fundamental role in glucose homeostasis [10]. It is reported that GK mRNA level is decreased in islets isolated from alloxan-treated mice [11]. Liver glucokinase (LGK) plays an essential role in controlling blood glucose levels and maintaining cellular metabolic functions, and is regulated by glucokinase regulatory protein (GKRP) [12]. Alloxan treatment led to reduction in glucokinase expression and enzymatic activity in the liver [13]. Until now, no one has reported the effect of phycocyanin on the plasma insulin level and insulin signaling pathway and GK expression in alloxan-induced diabetes mellitus mice.

In this study, we examined the antidiabetic therapeutic potential of phycocyanin using in vivo model. Moreover, we demonstrate that phycocyanin could ameliorate alloxan-induced diabetes mellitus in mice through insulin signaling and GK expression.

* Corresponding author.
E-mail address: ouyu2008@126.com (Y. Ou).

http://dx.doi.org/10.1016/j.cbi.2016.01.018
0009-2797/

2. Materials and methods

2.1. Chemicals

Phycocyanin was extracted and purified from *Spirulina platensis* as described previously [14]. The process mainly included homogenization, centrifugation, precipitation with ammonium sulphate, DEAE-Sepharose Fast Flow chromatography, hydroxylatite chromatography and Sephacryl S-200 chromatography. An absorbance ratio of $A_{620}/A_{280} > 4$ (electrophoretic purity) of phycocyanin was obtained. It was dissolved at a concentration of 20 mg/ml in PBS as a stock solution, stored at −80 °C and diluted before each experiment.

Alloxan were purchased from Sigma–Aldrich Chemical Company, USA. Metformin hydrochloride is the product of Beijing Jingfeng Pharmaceutical Co., Ltd., Beijing, China. Blood glucose, glycosylated serum protein (GSP) and glycated hemoglobin (HbA1c) test kits were purchased from Nanjing Jiangcheng Bioengineering Institute, Nanjing, China. Fasting serum insulin (FINS) test kit was purchased from Beijing North Institute of Biological Technology, Beijing, China. All the primary antibodies were purchased from Bioworld Technology, MN, USA. Horseradish peroxidase (HRP)-conjugated secondary antibody were purchased from MultiSciences, China. ECL plus kit was purchased from Beyotime Institute of Biotechnology, China. Prestained protein marker is the product of Fermentas, Thermo Scientific, USA.

2.2. Animals

Male ICR mice (18–22 g) were obtained from the Comparative Medical Center of Yangzhou University (Yangzhou, China) and maintained under standard conditions (12 h light–dark cycle; 23–25 °C; 35–60% r.h.). They were allowed one week to be quarantined and acclimated prior to experimentation.

2.2.1. Experimental induction of diabetes

ALX dissolved in sterile normal saline at a dose of 70 mg/kg body weight (intraperitoneal injection) was used to induce diabetes in the experimental mice after overnight fasting. After 72 h of ALX injection, the fasting blood glucose level was determined in the mice using blood glucose test kit. The blood glucose level above 11.1 mmol/L was considered to be diabetic and then used for the experiments as necessary.

2.2.2. Experimental design

Experimental design needed for the present in vivo study has been summarized as follows:

The mice were randomly assigned to six groups, each consisting of eight mice.

Group 1 (Control): normal mice received vehicle only.

Group 2 (Model): diabetic mice received vehicle only.

Group 3 (PC50): 4-week phycocyanin treatment orally at a dose of 50 mg/kg body weight after diabetic induction.

Group 4 (PC100): 4-week phycocyanin treatment orally at a dose of 100 mg/kg body weight after diabetic induction.

Group 5 (PC200): 4-week phycocyanin treatment orally at a dose of 200 mg/kg body weight after diabetic induction.

Group 6 (Metformin): given 4-week metformin orally at a dose of 300 mg/kg body weight after diabetic induction (used as positive control).

At the end of the 4-week period, the mice were fasted overnight (12 h), anaesthetized with 3% sodium pentobarbital (ip) and sacrificed by decapitation. Blood was placed into a centrifuge tube and allowed to clot to obtain the serum. The serum was separated by centrifugation at 1400 × g for 10 min and stored at −20 °C until assayed as described below. The liver and the pancreas were excised from the mice and stored at liquid nitrogen until use.

2.3. Biochemical analysis

The fasting blood glucose level in the micewas monitored at the end of every week during the treatment via tail prick method using blood glucose test kit. Fasting serum insulin (FINS) was determined using [^{125}I] insulin radioimmunoassay kit according to the guidelines indicated. Glycosylated serum protein (GSP) and glycosylated hemoglobin (GHb) were determined using commercial kits according to the enclosed guidelines.

2.4. Western blot analysis

The tissue (pancreas or liver) were resuspended in a lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1% NP-40, 0.1% SDS, and 1 mM PMSF), homogenized and clarified by centrifugation at 10,000 g for 10 min at 4 °C. Samples were resuspended in Laemmli buffer, separated electrophoretically by SDS-PAGE (10–12% gels), and subsequently transferred to 0.45 μm polyvinylidene difluoride (PVDF) membrane. Following transfer, membranes were first blocked for 1 h in TBST buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, and 0.15% Tween 20) containing 5% (w/v) nonfat milk powder, and then were incubated overnight at 4 °C with the appropriate specific primary antibodies diluted in TBST buffer. After washing, the membranes were incubated in the presence of horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Immunoreactive bands were detected by enhanced chemiluminescence using the ECL plus kit.

2.5. Statistical analysis

The significance of the data was determined using the Student *t*-test (two-tailed) or one-way analysis of variance.

P values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of phycocyanin on blood glucose levels in diabetic mice

The fasting blood glucose level in the mice was monitored every week (0, 1, 2, 3 and 4 weeks). The level of blood glucose in diabetic group showed significant increase compared to control group. Phycocyanin treatment decreased blood glucose levels in diabetic mice in both the time and dose-dependent manners. At the end of the experiment, doses of 100 and 200 mg/kg body weight of phycocyanin showed prominent effect (Fig. 1).

3.2. Effects of phycocyanin on glycosylated serum protein (GSP), glycosylated hemoglobin (GHb) and fasting serum insulin (FINS) levels of the mice

Table 1 shows the GSP, GHb and FINS levels in each group. The GSP as well as GHb were significantly increased ($P < 0.01$), while FINS was significantly decreased ($P < 0.01$) in diabetic group as compared to normal control group. Phycocyanin administration decreased GSP as well as GHb and increased FINS levels markedly ($P < 0.05$ or $P < 0.01$), as compared to the untreated group.

3.3. Effects of phycocyanin on insulin signaling in the liver and pancreas in diabetic mice

To investigate a possible role of phycocyanin in the insulin