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Phycocyanin prevents hypertension and low serum adiponectin level in a rat model of metabolic syndrome

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ABSTRACT

Endothelial dysfunction is associated with hypertension, atherosclerosis, and metabolic syndrome. Phycocyanin is a pigment found in the blue-green algae, *Spirulina*, which possesses antihypertensive effect. In this study, we hypothesized that phycocyanin derived from *Spirulina* exerts antihypertensive actions by improving endothelial dysfunction in metabolic syndrome. Spontaneously hypertensive/NIH-corpulent (SHR/NDmcr-cp) rats were divided into 4 groups then fed a normal diet with or without phycocyanin (2500-, 5000-, or 10 000-mg/kg diet) for 25 weeks. At 34 weeks of age, although systolic blood pressure was not significantly different among groups, phycocyanin-fed groups exhibited a dose-dependent decrease in blood pressure. Serum levels of adiponectin and messenger RNA levels of adiponectin and CCAAT/enhancer-binding protein α in the adipose tissue of rats fed diets containing phycocyanin tended to be higher than those of rats fed a normal diet, but the differences were not statistically significant. Immunohistochemistry analysis showed a significant and positive correlation between aortic endothelial nitric oxide synthase (eNOS) expression levels, a downstream target of the adiponectin receptor, and serum adiponectin levels, although there were no significant differences in eNOS expression among groups. There was also no significant correlation between eNOS expression levels and systolic blood pressure. These results suggest that long-term administration of phycocyanin may ameliorate systemic blood pressure by enhancing eNOS expression in aorta that is stimulated by adiponectin. Phycocyanin may be beneficial for preventing endothelial dysfunction-related diseases in metabolic syndrome.

Abbreviations: ANOVA, analysis of variance; C/EBPα, CCAAT/enhancer binding protein α; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; HDL-C, high-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NO, nitric oxide; PCR, polymerase chain reaction; SHR/NDmcr-cp rat, spontaneously hypertensive/NIH-corpulent rat; TNF-α, tumor necrosis factor α.

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1. Introduction

Metabolic syndrome is a major global health concern that is increasing in prevalence as a consequence of the ongoing obesity epidemic [1]. This syndrome consists of a combination of cardiovascular risk factors including hyperglycemia, hypertension, and dyslipidemia accompanied by visceral obesity and is linked to insulin resistance and development of diabetes mellitus as well as to nonalcoholic fatty liver disease (NAFLD) [1]. Visceral obesity and insulin resistance that play a central role in the metabolic syndrome induce adipose tissue dysfunction and are associated with an altered adipocytokine profile including oversecretion of leptin, tumor necrosis factor α (TNF-α), and interleukin-6 or hyposecretion of adiponectin. Metabolic syndrome exacerbates atherosclerosis and systemic vascular disease [2].

Vascular endothelial dysfunction, defined as a reduced vasodilating response to endothelial stimuli, is associated with obesity and insulin resistance and is a critical factor in the development of atherosclerosis [3,4]. Increased inflammatory cytokines and oxidative stress that result from the development of the metabolic syndrome affect endothelial cells directly and impair endothelial function [3,4]. Elevated blood pressure, low plasma levels of high-density lipoprotein cholesterol (HDL-C), and elevated triglycerides are also known as independent vascular risk factors [2].

Spirulina, which belongs to the cyanobacteria group (blue green algae), has been used as a source of food for more than a thousand years because of its high-content protein as well as essential nutrients such as carotenoids, vitamins, and minerals [5,6]. *Spirulina* has been reported to have beneficial effects against NAFLD, oxidative stress, hyperglycemia, and hypercholesterolemia [7]. Torres-Duran et al [6] reported that *Spirulina* also possesses antihypertensive activity in humans. Furthermore, they studied its effects on vasomotor responses in aortic rings and proposed it to have antihypertensive activity in experimental models. The detailed mechanisms and active ingredient(s) that mediate these effects, however, are unclear. Phycocyanin is a protein-bound pigment found in *Spirulina* that is marketed as a colorant for foods and cosmetics in Japan [8]. Several studies have previously reported to have a number of biological activities including antioxidative and antiinflammatory [8–10], antiplatelet [11], hepatoprotective [12], and cholesterol-lowering properties [13] in vivo or in vitro. Hitherto, there are no experimental reports on the effect of phycocyanin on hypertension or metabolic syndrome.

In this study, we hypothesized that phycocyanin derived from *Spirulina* exerts antihypertensive actions by improving endothelial dysfunction in metabolic syndrome. Therefore, the purpose of this study was to evaluate the effects of phycocyanin on metabolic syndrome including systemic hypertension and vascular diseases in a genetic animal model of metabolic syndrome, the spontaneously hypertensive/NIH-corpulent (SHR/NDmcr-cp) rats.

2. Methods and materials

2.1. *Animals and experimental design*

Nine-week-old male SHR/NDmcr-cp rats (n = 28) were purchased from Japan SLC Inc. (Hamamatsu, Japan). The rats were housed individually and were maintained at 22°C to 24°C with 50% to 60% relative humidity and a 12-hour dark/light cycle. All rats were acclimatized for 7 days during which they had access to a normal diet (MF; Oriental Yeast Co, Ltd, Tokyo, Japan) and water ad libitum. MF is formulated to provide adequate growth for rats according to the manufacturer's information. After acclimation, rats were randomly divided into 4 groups (7 rats/group): control group, fed a diet of MF; low (low-dose) group, fed MF containing phycocyanin (2500 mg/kg diet); middle (middle-dose) group, fed MF containing phycocyanin (5000 mg/kg diet); and high (high-dose) group, fed MF containing phycocyanin (10 000 mg/kg diet). Phycocyanin was obtained from DIC Lifetec Co, Ltd (Tokyo, Japan). The proximate analysis and phycocyanin content of the diets fed to rats are shown in Table 1. To ensure that there was no significant difference in body weight among groups, daily food intake was maintained at 32 g from 31 to 34 weeks of age in the middle group. The rats in all groups had free access to food and water at all other times throughout the study. Daily energy intake and body weight were monitored throughout the study. Systolic blood pressure was measured in 34-week-old conscious rats by the indirect tail-cuff method (BP MONITOR RATS & MICE Model MK-2000A; Muromachi Kikai Co, Ltd, Tokyo, Japan).

Table 1 – Proximate analysis and phycocyanin content of the diets fed to rats

Ingredient (g/100 g)	Control group (MF [a])	Low group (MF [a] with 0.25% phycocyanin)	Middle group (MF [a] with 0.5% phycocyanin)	High group (MF [a] with 1.0% phycocyanin)
Water	7.700	7.681	7.662	7.623
Crude protein	23.600	23.541	23.482	23.364
Crude lipid	5.300	5.287	5.274	5.247
Crude ash	6.100	6.085	6.070	6.039
Crude fiber	2.900	2.893	2.886	2.871
Nitrogen-free extract	54.400	54.264	54.128	53.856
Phycocyanin [b]	0.000	0.250	0.500	1.000
Total	100.000	100.000	100.000	100.000
Energy (kcal/100 g)	360.000	360.100	360.200	360.400

[a] MF is a normal diet provided by Oriental Yeast Co, Ltd.
[b] Phycocyanin is expected to have 400 kcal/100 g.