Gene 533 (2014) 57–66

Contents lists available at ScienceDirect

Gene

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

Antioxidant capacity responsible for a hypocholesterolemia is independent of dietary cholesterol in adult rats fed rice protein

Jixiang Cai [a], Lin Yang [a,*], Hong-Juan He [b], Tong Xu [c], Hong-Bo Liu [b], Qiong Wu [b], Ying Ma [a], Qiao-Hong Liu [a], Ming-Hao Nie [c]

[a] Department of Food Science, School of Food Science and Engineering, Harbin Institute of Technology, 73 Huanghe Road, Harbin 150090, China
[b] School of Life Science and Biotechnology, Harbin Institute of Technology, Harbin 150001, China
[c] Heilongjiang Provincial Hospital, Harbin 150036, China

ARTICLE INFO

Article history:
Accepted 30 September 2013
Available online 10 October 2013

Keywords:
Rice protein
Antioxidative response
Gene expression
Enzyme activity
Cholesterol
Adult rats

ABSTRACT

Dietary cholesterol and aging are major risk factors to accelerate oxidation process for developing hypercholesterolemia. The major aim of this study is to elucidate the effects of rice protein on cholesterol level and oxidative stress in adult rats fed with and without cholesterol. After 2 weeks of feeding, hepatic and plasma contents of cholesterol, reduced glutathione (GSH), oxidized glutathione (GSSG), malondialdehyde (MDA) and protein carbonyl (PCO) were measured. In liver, total antioxidative capacity (T-AOC), activities of antioxidant enzymes (total superoxide dismutase, T-SOD; catalase, CAT), glutathione metabolizing enzyme activities and gene expression levels (γ-glutamylcysteine synthetase, γ-GCS; glutathione reductase, GR; glutathione peroxidase, GPx) were determined. Under cholesterol-free/enriched dietary condition, T-AOC, activities of T-SOD and CAT, glutathione metabolism related enzymes' activities and mRNA levels (γ-GCS, GR and GPx) were effectively stimulated by rice proteins as compared to caseins. Compared with caseins, rice proteins significantly increased hepatic and plasma GSH contents, whereas hepatic and plasma accumulations of MDA, PCO and GSSG were significantly reduced by rice protein-feedings. As a result, the marked reductions of cholesterol in the plasma and in the liver were observed in adult rats fed rice proteins with and without cholesterol. The present study demonstrates that the hypocholesterolemic effect of rice protein is attributable to inducing antioxidative response and depressing oxidative damage in adult rats fed cholesterol-free/enriched diets. Results suggest that the antioxidant capability involved in the hypocholesterolemic action exerted by rice protein is independent of dietary cholesterol during adult period.

1. Introduction

The development of hypercholesterolemia is a multi-factorial process. Numerous studies indicate that increases in markers of oxidative stress are associated with elevated plasma cholesterol levels, suggesting that oxidative stress is one of major risk factors to cause hypercholesterolemia (Crawford et al., 2012; Schülke et al., 2012). Oxidative stress results from an imbalance between the production of free radicals and the scavenger antioxidant system (Malik and Storey, 2009). Accordingly, it becomes evident that oxidative stress can be suppressed by dietary antioxidant, highlighting a role of dietary component in improving oxidative stress to prevent the occurrence of hypercholesterolemia (Fang et al., 2002).

Rice is a staple cereal and widely consumed in the world (Nakamura et al., 2012; Ohtsubo and Nakamura, 2007; Tran et al., 2005; Yang et al., 2012a). There is a growing emphasis on improving knowledge of the physiological function of rice, in which the association of rice protein consumption with the reduction of cholesterol level has been extensively demonstrated in some studies (Jung et al., 2012; Kubota et al., 2013; Yang and Kadowaki, 2009; Yang et al., 2013a). Recently, Yang et al. reported that the strong protection by rice protein does against the risk of hypercholesterolemia and oxidative stress (Yang et al., 2012b). Results show that rice protein can improve oxidative stress through regulating glutathione metabolism and attenuating oxidative damage to lipids and proteins in growing rats, resulting in a hypocholesterolemic action. This study provides a novel insight into understanding the hypocholesterolemic mechanism exerted by rice protein, suggesting that rice protein can prevent the occurrence of hypercholesterolemia

Abbreviations: CAS, casein; CAS-C, cholesterol-supplemented casein; CAT, catalase; Cys, cysteine; γ-GCS, γ-glutamylcysteine synthetase; Glu, glutamate; Gly, glycine; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; Met, methionine; PCO, protein carbonyl; RP, rice protein; RP-C, cholesterol-supplemented rice protein; SAAs, sulfur amino acids; T-AOC, total antioxidative capacity; TC, total cholesterol; T-GSH, total glutathione; T-SOD, total superoxide dismutase.

* Corresponding author at: Department of Food Science, School of Food Science and Engineering, Harbin Institute of Technology, 73 Huanghe Road, Nangang District, Harbin 150090, Heilongjiang Province, China. Tel.: +86 451 8628 2910; fax: +86 451 8628 2906.
E-mail address: ly6617@hit.edu.cn (L. Yang).

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http://dx.doi.org/10.1016/j.gene.2013.09.124

in part through enhancing the antioxidative status. However, these observations are limited in growing rats fed with cholesterol-free diets. Thus, in view of these findings, a question raises whether rice protein can improve oxidative stress to modify the plasma cholesterol level in rats of other age as the addition of cholesterol to their diets.

The causes of the influence on oxidative stress appear to be complex and multi-factorial. Dietary cholesterol has a profound effect in the increase of oxidative stress. It is well established that the oxidation process can be accelerated by dietary cholesterol, resulting in an excessive production of free radicals in the face of defective anti-oxidant defenses (Mahfouz and Kummerow, 2000). Moreover, it has been demonstrated that higher level of cholesterol in their diets is a fatal factor to increase cholesterol levels in the plasma and liver (Balkan et al., 2004). Accordingly, dietary cholesterol has been suggested as one of causative factors that links oxidative stress with hypercholesterolemia. On the other hand, oxidative stress can be affected by aging process as well. Increasing evidences suggest that aging plays a mechanistic role in the acceleration of oxidative stress to produce hypercholesterolemia (Salmon et al., 2010). Inconsistent with this view, the fact that a stronger cholesterol-lowering effect of rice protein was found in adult rats than in young rats fed with cholesterol-free diets has been investigated by previous study (Yang et al., 2007). Furthermore, recent results showed that rice protein could produce a hypocholesterolemic effect in adult rats fed a cholesterol-enriched diet, indicating that the aging process and dietary cholesterol could not attenuate the hypocholesterolemic action induced by rice protein consumption (Yang et al., 2013b). However, up to now, we have limited knowledge base on whether, and how, rice protein can improve oxidative stress to regulate plasma cholesterol level during adult period.

Glutathione is a ubiquitous antioxidant in mammals, playing a critical role in protecting against oxidative stress through removal of many reactive species (Fang et al., 2002; Wu et al., 2004). The liver is a major site for glutathione metabolism, in which glutathione homeostasis is maintained by synthesis, oxidation and reduction (Forman et al., 2009; Wu et al., 2004). Several hepatic microsomal enzymes are implicated in modifying glutathione metabolism: γ-glutamylcysteine synthetase (γ-GCS), which is the rate limiting enzyme in glutathione synthesis; glutathione peroxidase (GPx), by which the major reaction of glutathione is reduction of hydroperoxides to yield glutathione disulfide; and glutathione reductase (GR), which reduces glutathione disulfide to glutathione (Forman et al., 2009; Wu et al., 2004). Thus, to elucidate the antioxidant mechanism exerted by rice protein, the influences of rice protein on glutathione metabolism-related enzymes should be taken into account.

The present study, therefore, was conducted to elucidate the relationship between plasma cholesterol level and oxidative stress in adult rats fed with rice protein. The key questions addressed are: (1) whether can rice protein induce antioxidant response to reduce plasma cholesterol level in adult rats fed with or without cholesterol in their diets and (2) how does rice protein modify glutathione metabolism to improve oxidative stress during adult period? In addition, the present work also focused on the influence of dietary cholesterol on the oxidative stress involved in modifying plasma cholesterol level.

2. Materials and methods

2.1. Protein sources

Rice protein (RP) from *Oryza sativa* L. cv. *Longjing* 21 (Rice Research Institute of Heilongjiang Academy of Agricultural Sciences, Jiamusi, China) and casein (CAS) (Gansu Hualing Industrial Group, Gansu, China) were used as the dietary protein sources in the present study. RP was prepared by the alkaline extraction method (Yang et al., 2011, 2012c).

2.2. Animals and diets

The present experiments were approved and carried out according to the "Rules for experiments on animals" published by Chinese Government (Beijing, China).

Adult male Wistar rats (body weight 390–410 g) were purchased from the Vital River Laboratories (Beijing, China) and individually housed in metabolic cages in a room maintained at 22 ± 2 °C under a 12 h light–dark cycle (07:00–19:00 for light). Rats were allowed free access to commercial pellets (Vital River Laboratories, Beijing, China) for 3 days. After acclimatization, rats were randomly divided into four groups of similar body weight. Each group consisted of 6 animals.

All animals were fed ad libitum with experimental diets according to the formula recommended by American Institute of Nutrition (Reeves et al., 1993). For 2 weeks, adult rats were fed 14% (as crude protein) dietary proteins without cholesterol (CAS and RP) with the addition of 1% cholesterol and 0.25% sodium cholate in their diets (CAS-C, cholesterol-supplemented casein; RP-C, cholesterol-supplemented rice protein). Diets were completed to 100% with starch. The composition of experimental diets is shown in Table 1.

2.3. Samples collection

During the feeding period, food consumption and body weight were recorded daily in the morning before replenishing the diets.

At the end of the feeding period, the rats were deprived for 18 h and then sacrificed. Blood was withdrawn from abdominal vein into a heparinized syringe under anesthesia with sodium pentobarbital (50 mg/kg body weight), immediately cooled on ice and separated by centrifugation at 12,000 × *g* for 5 min. The plasma obtained was frozen at −20 °C until analysis. After blood collection, the liver was excised immediately, rinsed in saline and weighed after blotted on a filter paper. The whole liver was cut into several portions and quickly freeze-clamped in liquid nitrogen and stored at −80 °C until analysis.

2.4. Analyses of cholesterols in plasma and liver

Plasma concentrations of total cholesterol (TC) were measured using the commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The total cholesterol in the liver were extracted and purified according to the method of Folch et al. (1957), and were measured with a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Table 1
Composition of experimental diets (g/kg).[a]

Ingredient	Cholesterol-free		Cholesterol-enriched	
	CAS	RP	CAS-C	RP-C
Casein[b]	160.2	–	160.2	–
Rice protein[c]	–	154.3	–	154.3
Sucrose	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0
Soybean oil	40.0	40.0	40.0	40.0
P-cornstarch	600.5	608.2	588.0	595.7
Mineral mix[d]	35.0	35.0	35.0	350
Vitamin mix[e]	10.0	10.0	10.0	0.0
Choline bitartrate	2.5	2.5	2.5	2.5
Tert-butylhydroquinone	0.008	0.008	0.008	0.008
L-Cystine	1.8	–	1.8	–
Cholesterol	–	–	10.0	10.0
Sodium cholate	–	–	2.5	2.5

[a] Experimental diets were accorded to AIN-93.
[b] Casein, protein concentration of 873.7 g/kg, obtained from Huanling Industrial Group (Gansu, China).
[c] Rice protein, protein concentration of 907.6 g/kg, prepared by our laboratory.
[d] Mineral mixture, AIN-93M-MX (Nosan Corp., Japan).
[e] Vitamin mixture, AIN-93-VX (Nosan Corp., Japan).