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Investigation of Blood Lipid-decreasing Effect of Resveratrol and Genetic-modified Rice on Mice

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ABSTRACT

Many resveratrol (Res) enriched genetic-modified (GM) fruits or crops were obtained through biotechnology. In this paper, the blood lipid-decreasing effects of exogenously supplemented Res and endogenously accumulated Res in rice grains were evaluated. The accumulation of triglyceride (TG) and cholesterol (CHO) induced by egg yolk emulsion in mice blood was suppressed by fenofibrate ($P < 0.01$) and purified Res ($P < 0.05$). However, the GM rice modified by peanut resveratrol synthase gene 1 (*PNRS1)* did not show a significant variance to wild-type (WT) rice. The possible reason might be that the content of Res in GM rice was hard to meet the usually used concentration of purified compounds. So, this fast evaluating method was limited to investigate the health care functions of some GM foods enriched active components in trace. Further attempts will be done to develop health functional rice of Res by genetic engineering to meet the growing needs of consumers.

Keywords : *Oryza sativa*, hyperlipidemia, triglyceride, cholesterol , health functional food

INTRODUCTION

Resveratrol, as a phytoalexin found in plant, has shown multi-functional effects on animals and human beings. The lifespan extension capacity of Res was reported in Yeast [1], Drosophila [2], *C. elegans* [3], Fly [4], Honeybee [5], and Mice [6]. Plenty of researches also reported the beneficial effects of Res in cancer prevention in several cancer cell lines [7,8], inflammation inhibition [9], and cardiovascular protection [10,11] in the aspects of health care, preventing and curing disease.

Res was shown the medicinal effects of leading to reducing lipid synthesis, increasing rates of fatty acid oxidation and preventing alcoholic liver steatosis [12]. Res has also been shown to attenuate high blood pressure and prevent cardiac hypertrophy in rats and mice [11]. When treated with procyanidin extracted from grape seed, the lipid and CHO metabolisms were altered between standard diet and high-fat diet fed hamsters [13]. To use Res in health functional foods [14], testing of GM products has also been performed on experimental animals, such as rats and mice. The rice of Cry1Ab protein contained mfb-MH86 was used to feed rats, and the results showed it is as safe and nutritious as the effects of non-GM rice [15]. The hearts of rats fed with Res modified tomato showed better cardiac performance, reduced myocardial infarct size and decreased number of apoptotic cardiomyocytes [10]. There was also research reporting that Res-enriched rice has more potent anti-metabolic syndrome activity than Res itself in mice by feeding high-fat diet [16].

For biosynthesis of Res, resveratrol synthase (RS) gene played a key role. It catalyzes coumaryl Co-A and malonyl Co-A to synthesize Res. Most of the plants contained the two substrates. But many food crops cannot synthesize Res because of the lacking RS gene [16]. In the previous study, a RS gene named *PNRS1* (GenBank: FM955393) was cloned from *Arachis hypogaea* [17]. The RS enzyme activity of *PNRS1* was confirmed in *Escherichia Coli* [18]. The binary vector of pCA1300-Ubi-PNRS1 was also conducted and used for transforming *PNRS1* into *Oryza sativa* [17]. Molecular identification and secondary metabolic detecting indicated the successful producing of endogenous Res enriched GM rice.

In this study, the grains of the Res enriched GM rice were used to feed the mice to evaluate their health care effects. Together with the blood lipid decreasing drug fenofibrate, the benefits of exogenously supplemented Res and endogenously accumulated Res to blood lipid decreasing were investigated in hyperlipidemia modelled mice. This research will provide reference for producing and providing better candidate resources of health functional food and point out the direction of optimization to further meet people's growing requirements of health care needs.

MATERIALS AND METHODS

GM rice

Res enriched rice was developed by transforming peanut RS gene of *PNRS1* into *Oryza sativa* cv. shengdao13 [17, 18]. The transgenic lines were cultivated for purification in the experimental fields. And a stable line L3 showing highest concentration of Res was selected for this experiment. Finally, about 5 kg seeds of wild-type and transgenic rice L3 were acquired for this experiment. Pretreatment of seeds with removing the seed coat and grounding the polished grains to fine powder were also performed.

Animals

Six-week-old Kunming mice (KM) of half male and half female were purchased from animal center of Shandong Lukang Pharmaceutical Co. (China). The male and female mice were caged individually. The mice were permitted access to food and water ad libitum. Specific pathogen free (SPF) folder of mice (Keaoxieli Inc., China) was the control diet. The mice were cultured in the animal room of Institute of Materia Medica, Shandong Academy of Chinese Medicine, under a 12 h light/12 h dark cycle at a temperature of 20-26℃ and humidity of 40-70%. Sixty KM mice were randomly divided into six groups, including normal control group (CK), negative control group (CK-), positive control group (CK+), external resveratrol treated group (RES), GM rice fed group (GM) and WT rice fed group (WT). Each group contained five male and five female mice.

Detection of *trans*-resveratrol

The content of Res in GM rice seeds was detected by high performance liquid chromatography (HPLC). The parameters of HPLC analysis were referred to Zheng et al. [18]. The trans-resveratrol (3,40,5-trihydroxy-trans-stilbene 99% GC; Sigma-Aldrich, St. Louis, MO) was employed as the standard sample. The amounts of Res were quantified by the corresponded peak areas.

Induction of the hyperlipidemia model

The treatments of each group were listed in Table 1. Fenofibrate (Laboratoires Fournier S. A., France) and *trans*-resveratrol standard (3,4′,5-trihydroxy-*trans*-stilbene, 99% GC; Sigma-Aldrich, St. Louis, MO) were supplemented to grain powder of WT rice to a final concentration of 1 g/kg. The rice grain powder was suspended with pure water at a final concentration of 0.5 g/ml. And 1 ml of this homogenate was used as an enema for each mouse twice per day. After two weeks of cultivation, hyperlipidemia models were induced by intraperitoneal injection of 75% hen egg yolk emulsion [19] in the mice of CK-, CK+, RES, GM, and WT group. The mice of CK group were injected with normal saline. Then, all the mice were fed for 12 h as usual, and then were fasted for 4 hours before blood sampling.

Table 1. The feeding experiments on the mice of each group.

Groups	Number of mice	Diet	Supplements (1 g/d)	Additive (1 g/kg)	Culture (weeks)	Injection (0.025 ml/g)	Intake of Res or Drug (mg/kg/d)
CK	10	SPF	NO[1]	NO	4	normal saline	0
CK-	10	SPF	NO	NO	4	egg yolk emulsion	0
CK+	10	SPF	WT rice	fenofibrate	4	egg yolk emulsion	50
RES	10	SPF	WT rice	Res	4	egg yolk emulsion	50
GM	10	SPF	GM rice	NO	4	egg yolk emulsion	0.05
WT	10	SPF	NO	NO	4	egg yolk emulsion	0

[1] NO nothing was supplemented or added.

Determination of triglyceride and cholesterol

To measure the blood lipid levels, the eyeballs of mice of each group were extracted and blood was drawn from the tail, and the serum was separated by centrifuging at 13,000 rpm for 10 min and immediately stored at -20℃ for further analysis. The levels of total TG and CHO in the serum were quantitatively determined by using an automatic biochemical analyzer (Hitachi 7180E; Japan) with reagents from Mike Biological Technologies Inc. (China).

Statistical analyses

All the data were recorded in Microsoft office excel 2010 for statistical analysis. The data was presented as mean ± standard deviation (SD). Student t-test was used to evaluate the variance and significance between each two of the experimental groups. The data of p value less than 0.05 was labeled.

RESULTS

Endogenous Res enriched GM rice foods

After HPLC detection, the concentration of *trans*-resveratrol was about 3 μg/g dry weight (DW) in the selected transgenic rice seeds [Fig. 1]. More, the Res content in GM rice polished grains was only 1 μg/g DW. While almost none of *trans*-resveratrol was identified in WT rice seeds and polished grains. This means that the *trans*-resveratrol was newly accumulated into GM rice. And the polished grains of GM rice were used as endogenous Res enriched functional foods for further analysis.

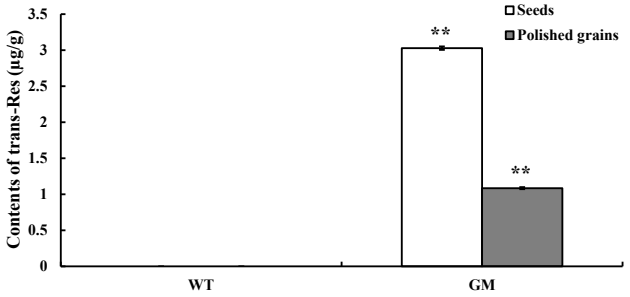

Fig. 1. The contents of *trans*-resveratrol in GM rice foods. *trans*-Res *trans*-resveratrol, WT wild-type rice, GM genetic-modified rice. ** indicates $p < 0.01$ compared to any one of the groups. The basic data has been reported by Zheng et al. previously (18).

Successful construction of hyperlipidemia model in mice

The levels of TG and CHO were used to appraise the accumulation of blood lipid [20]. So, the concentration of them was measured in serum of mice obtained from each experimental group. Almost all the mice were survived through the experiment, except that there was a death mouse in the CK+ and GM groups. The average levels of CHO and TG in each group were shown in Fig. 2A and Fig. 2B, respectively. The contents of TG and CHO in CK group were 2.412 ± 0.252 mmol/L and 1.004 ± 0.233 mmol/L, respectively. While there are 9.265 ± 1.873 mmol/L of CHO and 13.985 ± 3.744 mmol/L of TG in CK- group. Both levels of TG and CHO in CK- group were significantly higher than those in CK group ($p<0.01$). This indicates that the hyperlipidemia model in mice was successfully induced by the egg yolk emulsion.

External purified Res showing blood lipid reducing effect

The contents of TG and CHO in CK+ group were 6.262 ± 1.199 mmol/L and 8.851 ± 2.829 mmol/L, respectively. They were significantly lower ($p < 0.01$) than those in CK- group. This means that the lipid-decreasing control of fenofibrate was constructed successfully. Then, the function of exogenously supplemented Res and endogenously enriched Res in rice grain can be analyzed. The contents of TG and CHO in RES group are 7.487 ± 1.641 mmol/L and 10.008 ± 3.473 mmol/L, respectively. They were significantly lower ($P < 0.05$) than those in CK- group. This reveals that exogenously supplemented Res plays a role in decreasing of blood lipids.

No significant blood lipid decreasing effect observed for GM rice

However, the contents of TG and CHO showed no significant differences existed among CK-, GM, and WT groups. There were 9.965 ± 2.543 mmol/L of TG and 14.827 ± 4.380 mmol/L of CHO in GM group, and there are 10.072 ± 2.889 mmol/L of TG and 15.632 ± 4.670 mmol/L of CHO in WT group. This suggests that GM rice grains modified by peanut RS gene *PNRS1* do not meet a significant effect for blood lipid decreasing in this fast-evaluating experimental system.

DISCUSSION

Polyphenolic compounds are the important constituents of traditional health functional food, such as propolis [21], Red Wine [22], and Green tea [23]. The polyphenolic structure of Res also made it play a role in health care through involving in reactive oxygen species (ROS) related responses and regulating a series of ROS signal participated pathways [8,24,25]. To utilize this beneficial component, new type of health functional food was developed by genetic engineering. At present, various fruits, vegetables, and crops, such as in kiwifruits [26], apple [27], oilseed rape [28], pea [29], lettuce [30], tomato [10], potato [31], and rice [16,18] had been constructed the enrichment of Res through transgenic technology.

In this study, the purified Res showed a similar effect on blood lipid decreasing with fenofibrate in hyperlipidemia modelled mice (Fig. 2). The usually used supplementing dose of commercialized Res was 40 mg/kg to 320 mg/kg in previous researches [6, 11, 12]. And considered the dose of blood lipid decreasing drug fenofibrate, the final intake of purified Res was 50 mg/kg/d in this study. But the highest concentration of Res in the GM rice seeds was 3 μg/g, and the Res content in GM rice polished grains was only 1 μg/g [18]. This led to the final intake of Res in GM group was only 0.05 mg/kg/d (Table 1). Moreover, there was only 1.9 μg/g of Res in transgenic rice grain developed by Baek et al. [16]. And there was only 2.586 μg/g, 2.7 μg/g, 0.79–15.8 μg/g, and 56.4 μg/g of Res having been reported in transgenic grape [32], hops [33], tomato [10], and lettuce [30], respectively. So, there is still a long way to improve the contents of Res in GM products to reach an effective dose.

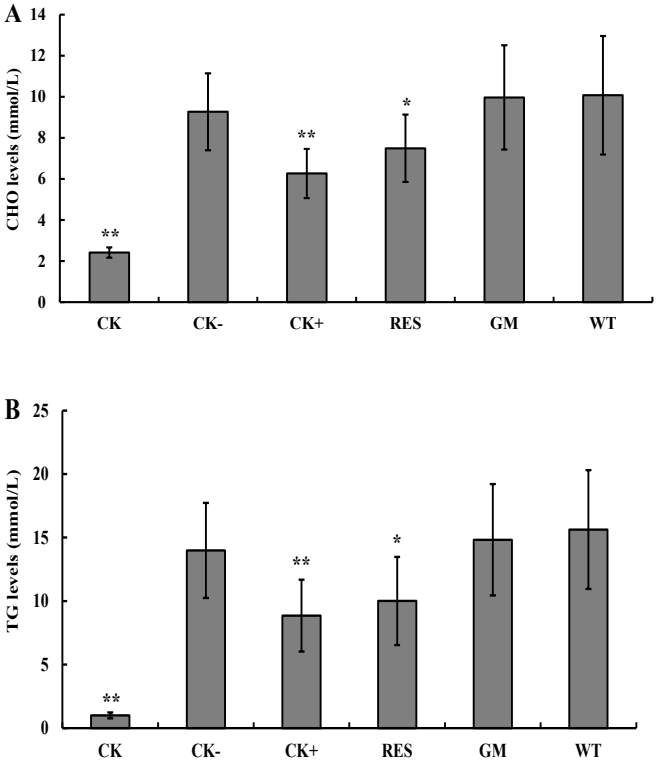

Fig. 2. Change of hyperlipidemia indexes in the mice with different treatments. **A** cholesterol (CHO) levels in the serum of mice; **B** triglyceride (TG) levels in the serum of mice. CK normal control group; CK- negative control group; CK+ positive control group treated by fenofibrate (50 mg/kg/d); RES external resveratrol (50 mg/kg/d) treated group; GM genetic-modified rice (0.15 mg/kg/d of Res) fed group; WT wild-type rice (0 mg/kg/d of Res) fed group (WT). Each group contained five male and five female mice Except the mice of CK group were injected with normal saline, the mice of other groups were intraperitoneally injected with 75% egg yolk emulsion to induce hyperlipidemia. Bars represent the mean values ± SD ($n \geq 9$). ** indicates $p < 0.01$, * indicates $p < 0.05$ compared to CK- group.

The mice fed with GM rice grains did not show a significant difference to those fed with wild-type rice grains after hyperlipidemia modelling (Fig. 2). This is mainly because of that the endogenous accumulation of Res in GM rice modified by heterogenous RS gene is still very low [16, 18]. And it cannot meet the effective dose as exogenously supplementing experiment usually used. Furthermore, the fast method by determining the levels of TG and CHO to evaluate the blood lipid-decreasing effect might be useful for the commercialized drug or purified Res. But there was only low dose of efficacious constituents in some health functional foods. The health care function of them depends on a relatively long term to express the accumulated effects.

The expression and enrichment of Res in GM rice over-expressing RS gene still need to be further optimized and screened to meet the customer needs. As Res showed multiple health care functions, the Res enriched GM food will attract more attention and apply in various of fields soon. Certainly, the actual benefits of Res-enriched diet to health care still need to be deeply and intensively studied before commercialization.

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