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In Vivo Antioxidant Activity of Lycopene from *Blakeslea Trispora* in Rats

Weilian Hu[1+], Dehui Dai[2], Wei Li[3]

[1] School of Biology and Chemical Engineering, Zhejiang University of Science and Technology, Hangzhou 310023, China;

[2] Zhejiang Academy of Medical Sciences, Zhejiang Center of Laboratory Animals，Hangzhou 310013, China;

Abstract. Lycopene, as a suspension in soybean oil, was tested for antioxidant activity by administration at 0, 267, 534, 1068 mg ₭g^{-1} body weight daily of groups of 12 male and 12 female rats for a period of 30 days. The lycopene examined in this study was derived from a fungal biomass (*Blakeslea trispora*). The results from this study showed that lycopene have the ability to reduce the level of malonaldehyde and increase the superoxide dismutase, catalase and glutathione peroxidase level when they were gavaged to rats up to 1068 mg ₭g^{-1} body weight daily, therefore we concluded that lycopene from *Blakeslea trispora* could enhance the antioxidation system in vivo and inhibit the generation of free radicals.

Keywords: Lycopene, antioxidation activity, rat, *Blakeslea trispora*

1. Introduction

Reactive oxygen species (ROS) are products of electron transport chains, enzymes, and redox cycling. Oxidative stress is a result of imbalance between the antioxidant defense system and the formation of ROS [1]. Oxidative stress may occur when ROS overwhelm the cellular antioxidant defense defenses, thus causing cell damage, death and exacerbate several age-related chronic diseases including cancer, Parkinson's disease and heart disease.

For aerobic organisms, they possess an antioxidant enzyme system as to terminate the propagation of free radical reactions, limit the formation of new free radicals, which includes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), to remove the ROS in cells [2]. Researchers also found that some exogenous dietary antioxidants may have the ability to reduce or terminate the propagation of free radical reactions, limit the formation of new free radicals, and therefore build a defense to protect the organism from the oxidative stress [3].

Nowdays, antioxidants are widely used in the food industry as potential inhibitors of lipid peroxidation [4]. However, those synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may accumulate in the body, resulting in liver damage and carcinogenesis [5]. In recent years, more and more attention has been paid to natural non-toxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases [6],[7].

Carotenoids are a family of fat-soluble pigments that are prevalent in numerous fruits and vegetables especially in tomato and carrot. In recent decades, many researchers have demonstrated that they have the potential at ameliorating oxidative stress [8]. Among the more than 600 different compounds founded in carotenoids, lycopene is proved to be the most prominent [9]. Researchers showed that lycopene plays an important role in health because of its highly efficient antioxidant scavenging activity against singlet-oxygen

+ Weilian Hu. Tel.: +86-571-85070370; Fax: ++86-571-85070378
E-mail address: weilian89@yahoo.com.

and free radicals [10]. This antioxidant activity is a potential mechanism by which lycopene may contribute to the prevention of a range of oxidative damage, toxicity, and disease [11], [12].

Although many studies had been conducted on the antioxidant properties of lycopene, most of these researches were focused on those lycopene produced by chemical synthesis or limited to those lycopene consisting of a stabilizing formulation matrix or fed along with a mixture of other carotenoids [13],[14]. The antioxidant study of lycopene derived from the fermentation of fungal has seldom been evaluated. The aim of current study was to evaluate its antioxidant properties by determine the SOD, CAT, GSH-Px and malonaldehyde (MDA) levels when the rats were administered at different dose of lycopene from *Blakeslea trispora.*

2. Material and Methods

2.1. Reagents

Superoxide dismutase (SOD), malondialdehyde (MDA), protein, glutathione peroxidase (GSH-Px), and catalase (CAT) test kits were purchased from Nanjing Jiancheng Bioengineering Institute (China); all other reagents were of analytical grade and made in China.

2.2. Preparation of lycopene

The test lycopene mycelium power was obtained from the fermentation of Blakeslea trispora in our lab. The content of lycopene, β-carotene and γ-carotene in the mycelium powder was 1.87%, 0.032% and 0.021%, respectively. As the ratio of β-carotene and γ-carotene in the mycelium powder was low, we considered the effect of mycelium powder was from the lycopene. 26.7 g mycelium powder was weighed into a 250 ml flask wrapped with aluminum foil to protect from light. 100 ml of soybean oil was added to the flask to dissolve carotenoids (the content of lycopene was 5 mg/ml). The mixture was stirred with magnetic stirrer for 30 min and then was kept at 4℃ until use. The dosing mixtures of the test substances were found to be stable following weekly preparation and storage in the refrigerator. This lycopene was diluted with soybean oil and the same sample was used in all treatments. Soybean oil was purchased in the local market.

2.3. Animals

Young male and female rats (48 rats/sex) were obtained from Zhejiang laboratory animal center under SPF conditions at Hangzhou, China. The age of the animals at study initiation was approximately 7 weeks. The weights of the animals were about 25-32g. The animals were quarantined and acclimatized for 1 week prior to the initiation of treatment. During the quarantine period, the animals were checked for health and normal growth. The rats were maintained in an air-conditioned room with a temperature range of 20-26℃, 40-70% relative humidity and a 12-h light/dark cycle, and had free access to standard laboratory chaw and tap water. Experiments on animals were performed according to animal ethics guidelines of Zhejiang laboratory animal center.

2.4. Experimental design

Rats were divided into four groups at random, each group has twenty-four with twelve male rats and twelve female rats. The rats were gavaged at the 0, 267, 534, 1068 mg ŀkg-1 b w daily for 30 days. Body weights were recorded weekly to adjust the administration level. After 30 days, the rats were fasted for 12 hours and then were sacrificed after taking blood sample from the orbit.

2.5. Treatment of brain, liver and skin

The brain, Liver and skin of rats were collected right after the rats were sacrificed, then the floating blood were washed out with ice-cold physiological saline, and the water was blotted up with filter paper, finally the tissues were weighed. Nine times of physiological saline were added and the mixtures were homogenized under centrifugation of 3000 rpm for 10 min at 5C, and then the supernatant liquid were collected and stored in 4C for the determination of SOD, CAT, MDA, GSH-Px level.

2.6. Statistical analysis

All statistical tests were performed with the statistical program SPSS 16.0. Oneway analysis of variance (ANOVA) was applied with Tukey's posthoc comparisons. The data were expressed as mean±SD in triplicate. Statistical significance was set at $P < 0.01$ and $P < 0.05$.

3. Result and Discussion

3.1. Effect of lycopene on SOD, MDA, CAT and GSH-Px in liver

The antioxidases in liver were affected by the lycopene. Table 1 showed the content of SOD, MDA, CAT and GSH-Px in liver when the rats were gavaged with lycopene for 30 days. As is shown in the table, the antioxidase such as SOD, CAT and GSH-Px levels in liver are higher in treated group than in control group, while MDA levels are lower in treated group than in control group. In the high dose group, the MDA level was significantly lower than the control ($P<0.01$), and the SOD, CAT and GSH-Px levels were improved significantly ($P<0.05$). The level of SOD and CAT were increased and the MDA was decreased in medium dose ($P<0.05$).

Table 1 Effect of lycopene on SOD, MDA, CAT and GSH-Px levels in liver

Group			Testing index			
			SOD (U mgprot^{-1})	MDA (nmol mgprot^{-1})	CAT (U mgprot^{-1})	GSH-Px (U mgprot^{-1})
Control			117.44±8.04	0.90±0.15	14.77±1.63	423.80±22.81
lycopene (mg kg^{-1}bw)	Low	267	122.72±11.59	0.81±0.17	15.83±1.33	434.52±18.31
	Medium	534	126.52±12.09*	0.78±0.13*	16.29±2.10*	438.17±21.85
	High	1068	131.47±12.87**	0.71±0.11**	17.12±3.53*	445.47±29.71*

Values are the mean±SD. $P < 0.01$ or $P < 0.05$ compared with control group.

3.2. Effect of lycopene on MDA, SOD, GSH-Px and CAT in brain

The antioxidases in brain were also affected by the lycopene. As is showed in table 2, administration of lycopene from fermentation of *Blakeslea trispora* can reduce the level of MDA and increase the SOD、CAT 、GSH-Px level in brain. There were significant different between of high dose group and the control group ($P<0.01$). The MDA was also reduced in the medium group ($P<0.01$) and low dose group ($P<0.05$), the GSH-Px level was significant increased in medium dose group ($P<0.05$).

3.3. Effect of lycopene on MDA, SOD, GSH-Px, CAT and Hpy in Skin

Table 2 Effect of lycopene on SOD, MDA, CAT and GSH-Px levels in brain

Group			Testing index			
			SOD (U mgprot^{-1})	MDA (nmol mgprot^{-1})	CAT (U mgprot^{-1})	GSH-Px (U mgprot^{-1})
Control			83.42±4.65	3.33±0.84	0.26±0.10	26.29±4.32
Lycopene (mg kg^{-1} bw)	Low	267	86.29±8.30	2.57±0.25*	0.30±0.08	28.14±1.77
	Medium	534	87.46±12.91	2.47±0.36**	0.35±0.14	30.48±3.17*
	High	1068	89.46±3.54**	2.43±0.19**	0.44±0.13**	32.10±3.47**

Values are the mean±SD. $P < 0.01$ or $P < 0.05$ compared with control group.

Table 3 Effect of lycopene on SOD, MDA, CAT and GSH-Px levels in skin

Group			Testing index			
			SOD (U mgprot^{-1})	MDA (nmol mgprot^{-1})	CAT (U mgprot^{-1})	GSH-Px (U mgprot^{-1})
Control			2.40±1.11	7.55±2.34	2.71±0.41	75.43±15.24
lycopene (mg kg^{-1} bw)	Low	267	3.18±1.91	5.82±3.40	2.45±0.29	77.40±4.29
	Medium	534	3.50±1.35*	5.55±2.32*	2.69±0.50	81.63±13.12
	High	1068	5.76±3.53**	4.14±2.07**	2.66±0.58	84.98±6.53*

Values are the mean±SD. $P < 0.01$ or $P < 0.05$ compared with control group.

The lycopene administration could lower MDA level and increase SOD、GSH-Px levels in rat skin, but have no effect on CAT level (Table 3). The SOD level of the high dose group is 1.4 times more than that of the control. From the comparison among activities of MDA in skin, it can be seen that the high dose ($P < 0.01$) and medium dose ($P < 0.05$) group have lower MDA activities than the control group.

Table 4 shows the effect of lycopene on Hyp level to female and male rats in skin. From the table, we found that the treatment of lycopene has no effect on Hyp level to female rats in skin, but high dose of lycopene can increase the Hyp level to male rats ($P<0.05$).

Table 4 Effect of lycopene on Hyp level in skin

Group		Hyp (ug·mg^{-1} skin)	
		Male	Female
Control		14.72±2.54	6.93±1.71
lycopene (mg·kg^{-1} bw)	Low 267	16.06±2.21	7.62±0.93
	Medium 534	16.32±1.20	8.20±1.13
	High 1068	17.66±2.36*	8.41±1.20

Values are the mean±SD. $P < 0.01$ or $P < 0.05$ compared with control group.

4. Discussion

There are two ways to make antioxidant activity come true. The first one is to reinforce antioxidant activity system in vivo and inhibit generation of free radicals; the second is to remove excess free radical directly [15], [16].

SOD is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defense against the toxic effects of superoxide radical in aerobic organisms. In this research, we found that SOD activities in all the organs significantly increased after high dose of lycopene was administered and this effect was associated with the decrease of MDA level. MDA was reported as the major marker of endogenous lipid peroxidation. We also found that the MDA was more sensitive than SOD to the treatment of lycopene, as low dose of lycopene administeration significantly decreased its level in all tissues. CAT is an enzyme located in peroxisomes and facilitates the removal of H_2O_2, which is metabolized to molecular oxygen and water [17]. The GSH-Px catalyses the reduction of H_2O_2 and lipid hydroperoxides at the expense of GSH. In this study, the CAT and GSH-Px levels are higher in treated group than in control group suggesting that in the treated groups, the antioxidant capacity was far more exceed than the amount of hydroperoxide products generated, thus we believe that lycopene could enhance the antioxidation system in vivo and inhibit the generation of free radicals.

5. Conclusion

In the current study, we found that the lycopene mycelium powder fermented from Blakeslea trispora have the ability to reduce the level of malonaldehyde and increase the superoxide dismutase, catalase and glutathione peroxidase level when they were gavaged to rats up to 1068 mg·kg-1 body weight daily, therefore we concluded that lycopene from Blakeslea trispora could enhance the antioxidation system in vivo and inhibit the generation of free radicals.

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