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Antioxidant acitivity *in vitro* and *in vivo* of the capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus*

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ABSTRACT

Crude capsule polysaccharides (CCP) were prepared from the culture of *Streptococcus equi* subsp. *zooepidemicus* C55129 and were partially purified through an anion-exchange column chromatography to afford partially purified capsule polysaccharides (PCP). The main component of CCP and PCP was hyaluronic acid. In vitro antioxidant assay, the capsule polysaccharides showed strong inhibition of lipid peroxidation and hydroxyl radical scavenging activity and moderate 1,1-diphenyl-2-picryldydrazyl radical scavenging activity. In addition, CCP exhibited much stronger reductive power than PCP. For antioxidant testing in vivo, CCP and PCP were orally administrated over a period of 15 days in a D-galactose induced aged mice model. As results, administration of capsule polysaccharides inhibited significantly the formation of malondialdehyde in mice livers and serums and raised the activities of antioxidant enzymes and total antioxidant capacity in a dose-dependent manner. However, the antioxidant activity of CCP was lower than that of PCP. The results suggest that the capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus* C55129 have direct and potent antioxidant activities.

1. Introduction

There are many kinds of reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radical and singlet oxygen. Although ROS at physiological concentration may be required for normal cell function, excessive amount of ROS can damage cellular components such as lipids, protein and DNA (Salvemini et al., 1999; Melov et al., 2000). It has been reported that oxygen-derived free radicals play an important role in decrepitude and may be important causative agents of senescence. And the free radical theory of aging suggests that the damage produced by the interactions of such free radicals with cellular macromolecules results in cellular senescence and aging (Rikans & Hornbrook, 1997; Wickens, 2001). Although almost all organisms possess antioxidant and repair systems to protect them against oxidative damage, these systems are insufficient to prevent the damage entirely. Therefore, a lot of attentions have been paid to antioxidants that play an important role in preventing senescence.

Polysaccharides, distributed widely in animals, plants and microorganisms, have been demonstrated to play an important role as dietary free radical scavenger in the prevention of oxidative damage in living organism (Tsiapali et al., 2001; Pang, Chen, & Zhou, 2000). And capsule polysaccharides of group A *Streptococcus* have been reported to have antioxidant activity (Cleary & Larkin, 1979). It has been reported that the main component of capsule polysaccharides from *Streptococcus equi* is hyaluronic acid (HA) (Woolcock, 1974). HA is very high molecular weight polymer made of repeating units of glucuronic acid (GlcA) and *N*-acetylglucosamine (GlcNAc) which is bounded by alternating β-(1 → 4)- and β-(1 → 3)-bonds. In recent years, several reports show that HA has antioxidant properties both in vitro and in vivo (Campo et al., 2004; Balogh, Illes, Szekely, Forrai, & Gere, 2003). And substantial evidences demonstrate that ROS are responsible for HA degradation in inflammatory diseases (Balogh et al., 2003). HA involvement in activation and modulation of the inflammatory response includes its antioxidant scavenging activity towards ROS, such as hydroxyl radical (·OH). The reduction of HA molar mass in the synovial fluid of patients suffering from rheumatic diseases has led to investigation of HA degradation in vitro by ROS (Šoltés et al., 2006). However, there is not any systematic investigation about the antioxidant activity of capsule polysaccharides from group C *Streptococcus zooepidemicus* both in vitro and in vivo. As we known, *Streptococcus equi* subsp. *zooepidemicus* is worldwide zoonotic pathogens, and the capsule polysaccharides produced may be effective to protect it against phagocytes during infection. The *Streptococcus equi* subsp. *zooepidemicus* is mainly isolated from pig in China, and it has pathogenicity only to pig but not to people. Therefore, it is quite different from the *Streptococcus zooepidemicus* strains which mainly isolated from horse in other worldwide areas.

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In addition, little information about its capsule polysaccharides and antioxidant activity is available. Therefore, evaluation of the antioxidant activity of the capsule polysaccharides produced by *Streptococcus equi* subsp. *zooepidemicus* will be important for the elucidation of function and utilization of the polymers.

The aim of present study is to evaluate the antioxidant activities of capsule polysaccharides produced by *Streptococcus equi* subsp. *zooepidemicus* C55129. Herein, we report in detail the antioxidant activities of capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus* C55129 by using D-galactose induced aged mice as in vivo model and different extracorporeal antioxidant methods.

2. Materials and methods

2.1. Reagents and materials

Strain of *Streptococcus equi* subsp. *zooepidemicus* C55129 was obtained from China Institute of Veterinary and Drug Control (Beijing, China). 1,1-Diphenyl-2-picryldydrazyl (DPPH), D-glucuronic acid sodium salt monohydrate and glucosamine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, USA). Assay kits for protein, malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and total antioxidant capacity (TAOC) were the products of Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The male Kunming mice were purchased from the Experiment Animal Center of Academy of Military Medical Sciences (Beijing, China). All other chemicals used were ultra pure or analytical grade.

2.2. Preparation of capsule polysaccharides

Streptococcus equi subsp. *zooepidemicus* C55129 was cultivated in medium (g/L) containing glucose, 40; yeast extract, 20; peptone, 10; K_2HPO_4, 2.5; NaCl, 2 and $MgSO_4{\cdot}7H_2O$, 1.0. The culture was operated at 37 °C with a shaking at 200 rpm for 24 h. The capsular polysaccharides were extracted from the culture according to the method described by Thonard (Thonard, Migliore, & Blustein, 1964) and further partially purified by DEAE Cellulose-52 anion-exchange chromatography. Briefly, the fermentation broth was centrifuged at 10,500g for 10 min, and the supernatant was precipitated by addition 2.5 volume of ethanol. The precipitate resulted was collected and dried to afford crude capsular polysaccharides (CCP). Then, CCP (50 mg) dissolved in de-ionized water was applied to a DEAE Cellulose-52 column (2.6 × 20 cm). The column was eluted with 0.5 M sodium chloride solution at a flow rate of 1 ml/min. And fractions (5 ml/tube) were collected automatically and checked by the carbazole method (Bitter & Muir, 1962). The fractions containing capsule polysaccharides were collected, concentrated, dialyzed and lyophilized to afford partially purified capsule polysaccharide (PCP).

2.3. Analysis of HA in capsule polysaccharides

To identify HA in the capsule polysaccharides, we carried out the carbazole assay (Bitter & Muir, 1962) and Elson Morgan's assay (Elson & Morgan, 1933) to determine the contents of GlcA and GlcNAc, respectively.

2.4. Determination of antioxidant activity of the capsule polysaccharides in vitro

2.4.1. Assay of reducing power and DPPH radicals scavenging ability

The reducing power of capsule polysaccharides was determined according to the method reported by Oyaizu (1986). The DPPH radical scavenging abilities of CCP and PCP were carried out according to our previous method reported (Ye, Wang, Zhou, Liu, & Zeng, 2008).

2.4.2. Determination of protective activity against Fe-induced lipid peroxidation and hydroxyl radical scavenging activity

The inhibition of lipid peroxidation of capsule polysaccharides was determined by quantification of the lipid peroxide decomposition product MDA based on reaction with thiobarbituric acid using egg yolk as oxydable substrate (Zhang & Yu, 1997). Hydroxyl radical scavenging activity of capsule polysaccharides was determined using the method reported by Smirnoff and Cumbes (Smirnoff & Cumbes, 1989).

2.5. Determination of antioxidant activity of capsule polysaccharides in vivo

2.5.1. Animal selection and experimental design

Male Kunming mice (8-weeks-old, 20 ± 2 g) were maintained in separated cages with distilled water. Under the conditions of 21 ± 1 °C and 50–60% relative humidity, they were free to access to food and water and kept on a 12-h light/dark cycles during the experiments. A total of 42 mice were equally divided into seven groups randomly including normal control group (NCG), D-galactose model control group (MCG), vitamin E positive control group (PCG), dose-dependent CCP groups (CCP 100, CCP 200, CCP 400) and PCP group (PCP 100). Mice in NCG were given 15 ml/kg (body weight) physiological saline solution (0.9% w/v) per day through subcutaneous injection and gastric gavage. Mice in MCG were fed with 15 ml/kg (body weight) physiological saline solution (0.9%, w/v) via gastric gavage and D-galactose injection (1.35 g/kg body weight) per day. Mice in PCG were given 100 mg/kg (body weight) vitamin E by gastric gavage and D-galactose injection (1.35 g/kg body weight) each day. The mice in dose-dependent capsule polysaccharide groups were given CCP (100, 200 and 400 mg/kg body weight) or PCP (100 mg/kg body weight) through gastric gavage and D-galactose injection (1.35 g/kg body weight) respectively, once a day for 15 consecutive days.

As to the methods of subcutaneous injection and gastric gavage, we described them briefly as following. For subcutaneous injection, we disinfected firstly the nape skin of rat for injection spot. Then we held a fold of skin with one hand, inserted the needle just under the skin at the base of the fold, and injected slowly the drug into rat. For gastric gavage, we held the rat very firmly by the skin of the neck and back so that the head of rat is kept immobile and in line with back. Then we put the feeding tube attached to the syringe into the mouth as far to one side as possible, and pushed gently the drug into the stomach after locating the entry to the esophagus. After pouring the drug, removed gently the feeding tube.

2.5.2. Assay of SOD, GSH-Px, MDA, CAT and TAOC

After overnight fasting following the last drug administration, the mice were sacrificed by decapitation. Blood samples were centrifuged at 4000g at 4 °C for 10 min to afford the serums required. The activities of SOD, CAT and GSH-Px and levels of MDA and TAOC in serum were measured according to the instructions on the kits.

The liver was excised, weighed and homogenized in 0.1 g/ml of ice-cold isotonic physiological saline based on wet weight. The suspension was centrifuged at 4000g at 4 °C for 10 min; the supernatant was subjected to the measurement of the activities of SOD, CAT and GSH-Px and the levels of MDA and TAOC.

2.5.3. Analysis of thymus and spleen indices

The spleen and thymus of the mice were also removed and weighed to afford the index of the spleen and thymus. The thymus and spleen indices were calculated according to the following formula:

Thymus or spleen index
= (weight of thymus or spleen/body weight) × 100

2.6. Statistical analysis

Data were statistically analyzed using the SPSS 16.0 software package by one-way ANOVA. Significant differences between two means were observed by Student-Newman-Keuls test. Differences were considered to be statistically significant if $P < 0.05$.

3. Results and discussion

3.1. Capsule polysaccharides from Streptococcus equi subsp. Zooepidemicus C55129

In the present study, CCP was extracted from the fermentation broth of *Streptococcus equi* subsp. *zooepidemicus* C55129 by centrifugation and ethanol precipitation. Furthermore, CCP was partially purified by DEAE Cellulose-52 anion-exchange chromatography to afford PCP. The percentage contents of GlcA and GlcNAc in CCP were 37.43 ± 0.95 and 33.51 ± 0.87, respectively, indicating a molar ratio of GlcA/GlcNAc 1.12. While, the percentage contents of GlcA and GlcNAc in PCP were 43.55 ± 1.03 and 40.14 ± 0.72, respectively, in a molar ratio of 1.08. As we know, HA is composed of GlcA and GlcNAc at a molar ratio of 1:1. The results demonstrated that the main component of capsule polysaccharides produced by *Streptococcus zooepidemicus* C55129 was HA, which was in parallel with that reported by Woolcock (Woolcock, 1974).

3.2. Antioxidant activities of capsule polysaccharides in vitro

3.2.1. Reductive potential of capsule polysaccharides

Various mechanisms, including reducing capacity, prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging have been reported to explain the antioxidant activities of polysaccharides (Diplock, 1997). The reductive capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Leskovar, Cantamutto, Marinangelli, & Gaido, 2004). Fig. 1A shows that the reductive potential of capsule polysaccharides exhibited a dose-dependent activity within the test concentration range of 150–1800 μg/ml. And the reductive powers of both CCP and PCP increased significantly ($P < 0.01$) with the increase of sample concentration. In addition, CCP showed a higher reducing power than PCP.

3.2.2. Scavenging effect on DPPH radicals

The DPPH free radical is a stable free radical, which has been widely accepted as a tool for estimating the free radical scavenging activities of antioxidants (Hu, Lu, Huang, & Ming, 2004). DPPH radical scavenging activity of the capsule polysaccharide was evident at all of the tested concentrations, but lower than that of ascorbic acid (Fig. 1B). The scavenging effect increased with the increase of concentration up to 1600 μg/ml. The highest DPPH radical scavenging activities were 46.83% and 52.24% for CCP and PCP, respectively, at the concentration of 1600 μg/ml. PCP showed stronger DPPH radical scavenging activity than CCP at the range of 200–1600 μg/ml ($P < 0.01$).

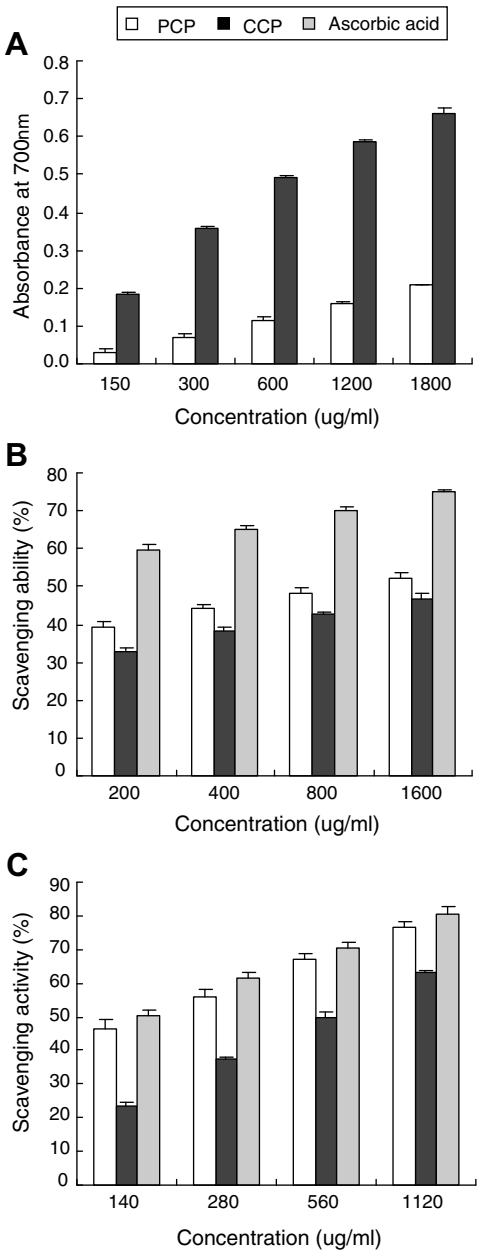

Fig. 1. The reductive potential (A), scavenging effect on DPPH radicals (B), scavenging activity against hydroxyl radical (C) of capsule polysaccharides from *Streptococcus zooepidemicus*. Data were presented as mean value ($n = 3$).

3.2.3. Hydroxyl radical scavenging activity of capsule polysaccharides

Hydroxyl radical and its subsequent radicals are the most harmful ROS and are mainly responsible for the oxidative injury of biomolecules. Fig. 1C showed the hydroxyl radical scavenging activities of capsule polysaccharides and ascorbic acid. Scavenging activity of CCP against hydroxyl radical was significantly lower than that of PCP at the range of 140–1120 μg/ml ($P < 0.05$). At a concentration of 1120 μg/ml, the scavenging rates of CCP, PCP and ascorbic acid were 63.0%, 76.6% and 80.4%, respectively. Similar results have been reported that HA isolated from rooster comb have strong hydroxyl radical scavenging activity in vitro (Balogh et al., 2003). Therefore, CCP and PCP showed strong hydroxyl radical scavenging activity. The antioxidant mechanism of capsule polysaccharides may be due to the degradation of the main component (HA) by hydroxyl radical (Li, Rosenfeld, Vilar, & Cowman, 1997).

3.2.4. Protective activity against Fe-induced lipid peroxidation

Lipid peroxidation is a common consequence of free radical-mediated chain reactions, some of its end-products such as lipid hydroperoxides and various species have unpaired electrons or the ability to attract electrons from other molecules. All of the end-products mentioned above can damage DNA directly or indirectly (Zhu et al., 2004). The effect of capsule polysaccharides on non-enzymatic peroxidation was shown in Fig. 2. In the egg yolk lipid peroxidation system, CCP and PCP inhibited lipid peroxidation in a concentration-dependent manner. The inhibitory rates of lipid peroxidation were 85.6% and 80.6% for PCP and CCP, respectively, at a concentration of 1120 μg/ml, indicating that both CCP and PCP possessed high inhibiting ability for lipid peroxidation. The mechanism of inhibiting effect may be relative to the polysaccharide-membrane interactions (Vereyken, Chupin, Demel, Smeekens, & De Kruij, 2001).

3.3. The antioxidant activity of capsule polysaccharides in vivo

3.3.1. Effect of the capsule polysaccharides on the activities of antioxidant enzymes in livers in aging mice

As shown in Table 1, a marked increase in MDA levels ($P < 0.01$) and decrease of antioxidant enzymes activities (SOD, GSH-Px, CAT) ($P < 0.05$) and TAOC ($P < 0.01$) were observed in livers between the treatments of NCG and MCG. Capsule polysaccharides and vitamin E treatment inhibited significantly the formation of MDA in mice livers and raised the activities of antioxidant enzymes and the level of TAOC in a dose-dependent manner (groups III–VII). In addition, the antioxidant activity of PCP was stronger than that of CCP at an identical dose of 100 mg/kg body weight.

3.3.2. Effect of the capsule polysaccharides on the activities of antioxidant enzymes in serums in aging mice

As shown in Table 2, administration of capsule polysaccharides and vitamin E dose-dependently elevated the activities of antioxidant enzymes and TAOC level, while reduced the level of MDA in serums (groups III–VII). Likewise, PCP exhibited stronger antioxidative effects than CCP at an identical dose of 100 mg/kg body weight.

A vast number of evidence implicates that aging is associated with a decrease in antioxidant status and the age-dependent increase in lipid peroxidation is a consequence of diminished antioxidant protection (Hagihara, Nishigaki, Maseki, & Yagi, 1984). The major antioxidant enzymes, including SOD, GSH-Px and CAT, are regarded as the first line of the antioxidant defense system against ROS generated in vivo during oxidative stress. SOD dismutates superoxide radicals to form hydrogen peroxide, which in turn is decomposed to water and oxygen by GSH-Px and CAT, thereby preventing the formation of hydroxyl radicals (Yao et al., 2005). Therefore, these enzymes act cooperatively at different sites in the metabolic pathway of free radicals. Many studies have shown that one or more of the antioxidant enzymes decrease as a consequence of aging (Inal, Kanbak, & Sunal, 2001). In the present study, we found that SOD, GSH-Px and CAT activities decreased markedly with aging and these changes had statistical significance in livers and serums. It is likely that the decrease in the activities of SOD and GSH-Px is the main factor in lipid peroxidative damage. In addition, the aging-related decrease in TAOC level suggests that the decrease in the non-enzymatic antioxidant defense probably also contributes to endogenous lipid peroxidation.

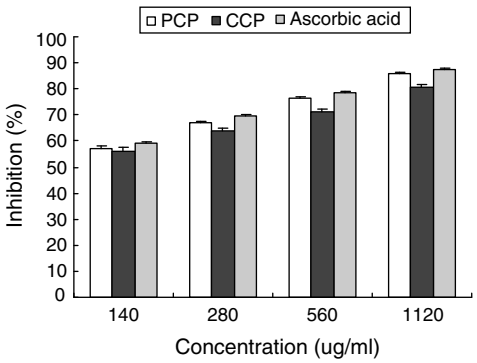

Fig. 2. Inhibition by capsule polysaccharide from *Streptococcus zooepidemicus* of $FeSO_4$ induced lipid peroxidation of egg yolk with ascorbic acid as a positive control. Data were presented as mean value ($n = 3$).

The capsule polysaccharides showed inhibiting ability for lipid peroxidation as observed in the reduction of MDA production in aging mice. The result was consistent with that of capsule polysaccharides *in vitro*. The administration of capsule polysaccharides induced the activities of SOD, GSH-Px and CAT and increased TAOC level in aging mice. The enhanced activities of SOD, GSH-Px and CAT and increased TAOC level in the aging mice could be very effective in scavenging various types of oxygen free radicals and their products. So the inhibitory effect of the capsule polysaccharides on lipid peroxidation might be attributed to their effects on the antioxidant enzymes and non-enzymatic system. Although the antioxidant mechanism of the capsule polysaccharide is not clear to date, it is possible that the effects of the capsule polysaccharides on SOD, GSH-Px and CAT are associated with the induction on gene expression of SOD, GSH-Px and CAT (Pang et al., 2000). It has been reported that scission of HA can occur by free radicals under oxidative conditions (Deguine et al., 1998). Therefore, it is possible that the main component (HA) of the capsule polysaccharides is degraded by ROS, and the capsule polysaccharides scavenge directly ROS during the degradation. The low molecular weight fragments of HA produced might also attribute to the ROS scavenger activity (Trabucchi et al., 2002). In addition, different molecular sizes of HA can induce a number of antioxidant enzymes (SOD, GSH-Px, CAT) and inhibit lipid peroxidation due to its stimulating translational process and/or post-translational process of these antioxidant enzymes.

3.3.3. Effect of the capsule polysaccharides on thymus and spleen index in aging mice

As shown in Table 3, the thymus and spleen indices were significantly decreased for MCG ($P < 0.01$) compared with NCG. Administration of capsule polysaccharides and vitamin E seemed to have remarkable effects on increasing the two indices in immune organ of mice in a dose-dependent manner (groups III–VII). At a dose of 100 mg/kg body weight, the spleen index of PCP was higher than that of CCP.

A variety of immune changes occur in both animals and humans with increasing age. The aging of the immune system (immunosenescence) is associated with dramatic reductions in immune responsiveness as well as functional dysregulation. As shown in Table 3, the decrease in thymus and spleen indices in aged mice is a good indicator of age-induced decline in immune function. The administration of capsule polysaccharides enhanced spleen index and thymus index in aging mice, suggesting that the capsule polysaccharides stimulated effectively the immune system of aged mice. Although the exact mechanism for the immune-stimulating activity of the capsule polysaccharides is not known, it is proposed that they may act by inducing a number of antioxidant enzymes (SOD, GSH-Px, CAT) and decreasing lipid peroxidation. It has been reported that HA fragments can stimulate immune system (Horton, Shapiro, Bao,

Table 1
Effects of capsule polysaccharides on the activities of SOD (U mg^{-1} protein), CAT (U mg^{-1} protein), GSH-Px (U mg^{-1} protein), TAOC (U mg^{-1} protein) and levels of MDA (nmol mg^{-1} protein) in livers in aging mice[a]

	Group I (NCG)	Group II (MCG)	Group III (PCG 100)	Group IV (CCP 100)	Group V (CCP 200)	Group VI (CCP 400)	Group VII (PCP 100)
SOD	146.28 ± 32.64	119.28 ± 6.05[d]	225.90 ± 22.46[c]	178.71 ± 15.156[b]	217.91 ± 26.40[c]	211.48 ± 22.09[c]	238.70 ± 11.06[c]
CAT	81.20 ± 7.01	61.34 ± 5.12[d]	80.96 ± 5.13[b]	78.29 ± 9.61[b]	81.44 ± 10.61[b]	98.87 ± 17.12[c]	92.82 ± 10.83[c]
GSH-Px	607.44 ± 9.90	447.42 ± 28.96[d]	763.01 ± 60.77[c]	540.85 ± 63.39	609.67 ± 75.59[b]	705.89 ± 58.04[c]	839.18 ± 124.15[c]
TAOC	1.93 ± 0.10	1.50 ± 0.15[e]	1.90 ± 0.19[c]	1.59 ± 0.07	1.74 ± 0.16[b]	1.75 ± 0.11[b]	1.95 ± 0.05[c]
MDA	1.39 ± 0.36	2.15 ± 0.35[e]	1.05 ± 0.34[c]	1.05 ± 0.24[c]	0.88 ± 0.11[c]	0.89 ± 0.05[c]	1.30 ± 0.30[c]

[a] Data were presented as means ± SD ($n = 6$) and evaluated by one-way ANOVA followed by the Student-Newman-Keuls test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$.
[b] $P < 0.05$, compared with model group.
[c] $P < 0.01$, compared with model group.
[d] $P < 0.05$, compared with normal group.
[e] $P < 0.01$, compared with normal group.

Table 2
Effect of capsule polysaccharides on the activity of SOD (U ml^{-1}), CAT (U ml^{-1}), GSH-Px (U ml^{-1}), TAOC (U ml^{-1}) and levels of MDA (nmol ml^{-1}) in serums in aging mice[a]

	Group I (NCG)	Group II (MCG)	Group III (PCG 100)	Group IV (CCP 100)	Group V (CCP 200)	Group VI (CCP 400)	Group VII (PCP 100)
SOD	123.30 ± 3.82	102.75 ± 3.89[e]	107.38 ± 4.78	124.75 ± 2.47[c]	128.80 ± 2.55[c]	122.30 ± 2.46[c]	130.10 ± 5.53[c]
CAT	38.69 ± 2.65	31.49 ± 4.23[d]	38.82 ± 1.67[b]	37.69 ± 0.44	42.48 ± 1.44[c]	46.97 ± 4.28[c]	47.90 ± 3.80[c]
GSH-Px	1182.41 ± 115.46	864.71 ± 90.93[d]	1176.59 ± 34.61	1388.21 ± 158.06[c]	1647.10 ± 107.87[c]	1764.71 ± 50.50[c]	1882.41 ± 115.47[c]
TAOC	23.93 ± 1.31	16.03 ± 1.46[e]	24.73 ± 1.03[c]	17.45 ± 2.05	18.56 ± 2.21	25.10 ± 2.97[c]	22.45 ± 2.05[b]
MDA	24.00 ± 2.66	41.08 ± 2.94[e]	20.39 ± 0.54[b]	15.154 ± 2.90[c]	16.42 ± 3.26[c]	21.69 ± 2.39[c]	17.39 ± 0.65[c]

[a] Data were presented as means ± SD ($n = 6$) and evaluated by one-way ANOVA followed by the Student-Newman-Keuls test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$.
[b] $P < 0.05$, compared with model group.
[c] $P < 0.01$, compared with model group.
[d] $P < 0.05$, compared with normal group.
[e] $P < 0.01$, compared with normal group.

Table 3
Effects of capsule polysaccharides on the thymus and spleen indices in different organs in aging mice[a]

	Group I (NCG)	Group II (MCG)	Group III (PCG 100)	Group IV (CCP 100)	Group V (CCP 200)	Group VI (CCP 400)	Group VII (PCP 100)
Spleen index	0.347 ± 0.069	0.206 ± 0.026[e]	0.314 ± 0.037[c]	0.266 ± 0.047	0.282 ± 0.010[b]	0.328 ± 0.059[c]	0.307 ± 0.051[c]
Thymus index	0.390 ± 0.018	0.229 ± 0.016[e]	0.350 ± 0.019[c]	0.430 ± 0.056[c]	0.360 ± 0.048[c]	0.347 ± 0.042[c]	0.310 ± 0.054[c]

[a] Data were presented as means ± S.D. (n = 6) and evaluated by one-way ANOVA followed by the Student-Newman-Keuls test to detect inter-group differences. Differences were considered to be statistically significant if $P < 0.05$.
[b] $P < 0.05$, compared with model group.
[c] $P < 0.01$, compared with model group.
[e] $P < 0.01$, compared with normal group.

Lowenstein, & Noble, 1999; Slevin, Kumar, & Gaffney, 2002). Therefore, it is possible that the main component (HA) of capsule polysaccharides and/or HA fragments of different molecular size stimulated the immune mechanism and induced a number of antioxidant enzymes (SOD, GSH-Px, CAT) and decreased lipid peroxidation.

4. Conclusions

In the present study, the antioxidant properties of capsule polysaccharides from *Streptococcus equi* subsp. *zooepidemicus* C55129 were demonstrated by using a variety of testing systems in vitro and by a D-galactose induced aging mice model in vivo. In vitro antioxidant assay, CCP exhibited stronger antioxidant activities than PCP. In vivo antioxidant testing, both CCP and PCP reduced lipid peroxidation accelerated by age-induced free radicals, but the antioxidant activity of CCP was lower than that of PCP. The results suggest that the capsule polysaccharides from *Streptococcus equi* subsp. zooepidemicus C55129 have direct and potent antioxidant activities. As the main component of capsule polysaccharides is HA, further works on function evaluation and utilization of the capsule polysaccharides are in progress.

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