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Selenylation modification can enhance immune-enhancing activity of Chinese angelica polysaccharide

Tao Qin[a,1], Jin Chen[b,1], Deyun Wang[a], Yuanliang Hu[a,*], Jing Zhang[a], Mi Wang[a], Shulei Qiu[a], Zhenzhen Gao[a], Rongrong liu[a], Yun Yu[a], Yee Huang[a], Qiaochu Wang[c], Qiaoxiu Wang[a]

[a] Institute of Traditional Chinese Veterinary Medicine, College of Veterinary Medicine, Nanjing Agricultural University, Nanjing 210095, PR China
[b] National Research Center of Veterinary Biological Engineering and Technology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, PR China
[c] Department of Biotechnology, School of Basic Medical Sciences, Nanjing Medical University, Nanjing 210029, PR China

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ABSTRACT

On the basis of previous test that selenizing Chinese angelica polysaccharides (sCAPs) with stronger immune-enhancing activity in vitro were picked out, the immune-enhancing activity in vivo of three sCAPs, $sCAP_2$, $sCAP_6$ and $sCAP_8$, at high and low dosage were compared taking the unmodified Chinese angelica polysaccharide (CAP) as control by determination of peripheral lymphocyte proliferation, serum antibody titer, IFN-γ and IL-6 contents in chicken vaccinated with Newcastle Disease vaccine. The results showed that three sCAPs at suitable dosage could significantly promote lymphocyte proliferation, enhance serum antibody titer, IFN-γ and IL-6 contents as compared with unmodified CAP, $sCAP_2$ at low dosage possessed the strongest action. These results indicated that selenylation modification could significantly enhance the immune-enhancing activity of CAP, $sCAP_2$ possessed the best efficacy and would be as a component drug of new-type immunoenhancer.

1. Introduction

Polysaccharides and polysaccharides complexes are able to activate the activity of immune system and enhance the impact on specific, non-specific immunity, cellular and humoral immunity of organism (Day & Sheehan, 2001; Fukui, Feizi, Galustian, Lawson, & Chai, 2002; Grobe et al., 2001). Many researches found that appropriate molecular modification or structure reform could make polysaccharides generate new activity or further enhance original activity (Chihara, Maeda, Hamura, Sasaki, & Fukuoka, 1969; Melo, Feitosa, Freitas, & Paula, 2002; Wang & Zhang, 2009; Zhang, Cheung, Ooi, & Zhang, 2004). At present, people pay more attention to the selenylation modification of polysaccharides. The most commonly used method is Nitric acid–sodium selenite (HNO_3–Na_2SeO_3) method since it is characterized by simpler reaction conditions and shorter duration and higher selenium content in the product and so on.

In our previous researches, Chinese angelica polysaccharide (CAP) was extracted by water decoction and ethanol precipitation, purified through eliminating protein by Sevage method and column chromatography of Sephadex G-200, and selenizingly modified by HNO_3–Na_2SeO_3 method according to $L_9(3^4)$ orthogonal design of three-factors, the amount of sodium selenite (Na_2SeO_3), reaction temperature and reaction time each at three levels. Nine selenizing CAPs (sCAPs) named $sCAP_1$–$sCAP_9$ were obtained. Their effects on chicken peripheral lymphocytes proliferation in vitro were compared by MTT assay taking the unmodified CAP as control. The results showed that selenylation modification could improve the immune-enhancing activity in vitro of CAP, $sCAP_2$, $sCAP_6$ and $sCAP_8$ presented stronger immune-enhancing activity (Qin et al., 2013).

In this research the immune-enhancing activities in vivo of $sCAP_2$, $sCAP_6$ and $sCAP_8$ at high and low dose were compared taking the unmodified CAP as control by the determination of peripheral lymphocyte proliferation, serum antibody titer, IFN-γ and IL-6 contents in chicken vaccinated with Newcastle Disease vaccine taking unmodified CAP as control. The purpose of this research further validate the potential of selenylation modification to enhance the immune-enhancing activity of CAP, pick out optimal selenizing

Abbreviations: CAP, Chinese angelica polysaccharide; sCAP, selenizing Chinese angelica polysaccharide; HNO_3, nitric acid; Na_2SeO_3, sodium selenite; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PHA, phytohemagglutinin; CMF, calcium and magnesium-free; PBS, phosphate-buffered saline; DMSO, dimethyl sulfoxide; IFN-γ, Interferon γ; IL-6, Interleukin 6.

* Corresponding author. Tel.: +86 25 84395203; fax: +86 25 84398669.
E-mail addresses: ylhu@njau.edu.cn, ylhu@sohu.com (Y. Hu).

[1] These authors contributed equally to this work.

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polysaccharide and its dose, and establish the foundation for development of new-type polysaccharides immunoenhancer.

2. Materials and methods

2.1. Vaccine and reagents

Newcastle Disease vaccine (La Sota strain, No. 119076) was purchased from Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Science.

RPMI-1640 (GIBCO) supplemented with 100 IU mL^{-1} benzylpenicillin, 100 IU mL^{-1} streptomycin and 10% fetal bovine serum was used for washing and re-suspending cells, diluting mitogen and culturing the cells. Phytohemagglutinin (PHA, Sigma), as a T-cell mitogen, was dissolved into 0.1 mg mL^{-1} with RPMI-1640. Hanks' solution, pH was adjusted to 7.4 with 5.6% sodium bicarbonate solution, supplemented with benzylpenicillin 100 IU mL^{-1} and streptomycin 100 IU mL^{-1}, was used for diluting blood. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, American Co.) was dissolved into 5 mg mL^{-1} with calcium and magnesium-free (CMF) phosphate-buffered saline (PBS, pH 7.4). Sodium heparin was dissolved into 5 mg mL^{-1} with PBS. These reagents were filtered through a 0.22 μm syringe filter. PHA and sodium heparin solution were stored at −20 °C, the others were at 4 °C and MTT solution was in dark bottle.

Dimethylsulfoxide (DMSO), Nitric acid (HNO_3) and Sodium selenite were the products of Shanghai Lingfeng Chemical Reagent Ltd., No. 060902. Lymphocytes Separation Medium (No. 20110923) was manufactured by Shanghai Hengxin Chemicals Ltd.

Chickens Interferon γ (IFN-γ) ELISA kits and Chickens Interleukin 6 (IL-6) ELISA kits were the products of Shanghai Langdun Biotechnology Inc.

2.2. Preparation of sCAPs

CAP, $sCAP_2$, $sCAP_6$ and $sCAP_8$ were prepared in our laboratory (Qin et al., 2013). Briefly, CAP was extracted by water decoction and ethanol precipitation, purified through eliminating protein by Sevage method and column chromatography of Sephadex G-200 (2 cm × 100 cm), its carbohydrate content was 92.7% determined by the phenol-sulfuric acid method (Li & Wang, 2008; Yu, Yang, Liu, & Liu, 2009). Three selenizing polysaccharides ($sCAP_2$, $sCAP_6$ and $sCAP_8$) were prepared by Nitric acid–sodium selenite method (Li, Miu, & Liu, 2001) under the optimal modification conditions obtained in our previous researches: the usage amounts of sodium selenite were 200 mg, 300 mg and 400 mg, the reaction temperatures were 70 °C, 90 °C and 70 °C, the reaction times were 8 h, 10 h and 8 h, respectively. Their selenium contents were 12.98 mg g^{-1}, 10.66 mg g^{-1} and 6.41 mg g^{-1} measured by atomic fluorescence spectrometry method (Gao, Qin, & Huang, 2006) and carbohydrate contents were 50.9%, 57.2% and 63.2% measured by the phenol-sulfuric acid method, in turn. According to content of polysaccharides, $sCAP_2$, $sCAP_6$ and $sCAP_8$, were diluted into high (3 mg mL^{-1}) and low (1 mg mL^{-1}) concentrations, CAP, into 3 mg mL^{-1}, with PBS (pH 7.4). The diluted preparations were sterilized by pasteurization and tested the endotoxin. When the endotoxin amount was up to the standard of Chinese Veterinary Pharmacopeia (less than 0.5 EU mL^{-1}) (Veterinary Pharmacopoeia Commission of the People's Republic of China, 2000), they were stored at 4 °C for the test (Kong, Hu, Rui, Wang, & Li, 2004).

2.3. Animals and experimental design

One-day-old White Roman chickens (male) were purchased from Tangquan Poultry Farm. At 14-days-old, their average maternal ND-HI antibody titer was 2.8 log 2, 270 chickens were selected and randomly divided into 9 groups. The chickens except in blank control (BC) group were vaccinated with 0.5 mL of ND-IV vaccine, repeated vaccination at 28 days old. At the same time of the first vaccination, the chickens in high and low dosage of three sCAPs groups were intramuscularly injected respectively with 0.5 mL of $sCAP_2$, $sCAP_6$ and $sCAP_8$ at high and low concentrations, in CAP control group, with 0.5 mL of CAP, in vaccination control (VC) and BC group, with equal volume of physiological saline, once a day for three successive days.

2.4. Peripheral lymphocytes proliferation assay

On days 7 (D_7), 14 (D_{14}), 21 (D_{21}) and 28 (D_{28}) after the first vaccination, the bloods (5 mL) sample of four chickens randomly from each group were collected and transferred immediately into aseptic capped tubes with sodium heparin, then diluted with equal volume of Hanks' solution and carefully layered on the surface of lymphocytes separation medium. After 20 min of centrifuged at 2000 rpm, a white cloud-like lymphocytes band was collected and washed twice with RPMI-1640 media without fetal bovine serum. The resulting pellet was re-suspended to 2.5×10^6 mL^{-1} with RPMI-1640 media, inoculated into 96-well culture plates, 80 μL per well, then another 20 μL of PHA was added and each sample seeded 4 wells. The plates were incubated at 39.5 °C in a humid atmosphere of 5% CO_2. After 44 h of the incubation period, 20 μL of MTT (5 μg mL^{-1}) was added into each well, and continued to incubate for 4 h. The supernatant was removed carefully and 100 μL of DMSO were added into each well to dissolve the formazan crystals. The plates were shaken for 5 min to dissolve the crystals completely. The absorbance each well was measured by microliter enzyme-linked immunosorbent assay reader (Model DG-3022, East China Vacuum Tube Manufacturer) at a wave length of 570 nm (A_{570} value) (Wang et al., 2005) as the index of lymphocytes proliferation. At the same time the highest lymphocyte proliferation rates of all polysaccharide groups were calculated to compare the action strength of every polysaccharide according to the equation: The highest lymphocyte proliferation rate (%) = $(\bar{A}_{\text{polysaccharid group}} - \bar{A}_{\text{VC group}})/\bar{A}_{\text{VC group}} \times 100\%$ ($\bar{A}_{\text{polysaccharide group}}$ was the highest A_{570} value among four time points, $\bar{A}_{\text{VC group}}$ was the A_{570} value at the same time point with the polysaccharide group).

2.5. Serum HI antibody assay

On days 7 (D_7), 14 (D_{14}), 21 (D_{21}) and 28 (D_{28}) after the first vaccination, six chickens were sampled randomly from each group for examination of serum hemagglutination inhibition (HI) antibody titer by micro-method (Abula et al., 2011).

2.6. Serum IFN-γ and IL-6 contents assay

On days 14 (D_{14}), 21 (D_{21}) and 28 (D_{28}) after the first vaccination, six chickens were sampled randomly from each group for determining the serum contents of IFN-γ and IL-6 by Enzyme-linked Immunosorbent Assay (ELISA).

2.7. Statistical analysis

Data are expressed as means ± SD. Duncan's multiple range test was used to determine the difference among polysaccharides and control groups. Significant differences between means were considered at $P < 0.05$.

Table 1
The changes of lymphocyte proliferation in every group (A_{570} value).

Group	D_7	D_{14}	D_{21}	D_{28}
$sCAP_{2L}$	0.176 ± 0.001 [a]	0.185 ± 0.002 [b]	0.385 ± 0.002 [a]	0.311 ± 0.025 [a]
$sCAP_{2H}$	0.134 ± 0.002 [d]	0.146 ± 0.001[e]	0.285 ± 0.001 [ef]	0.253 ± 0.003 [cd]
$sCAP_{6L}$	0.142 ± 0.005 [c]	0.165 ± 0.001[c]	0.334 ± 0.011 [bc]	0.291 ± 0.004 [b]
$sCAP_{6H}$	0.162 ± 0.002 [b]	0.153 ± 0.001[cd]	0.311 ± 0.006 [d]	0.311 ± 0.007 [a]
$sCAP_{8L}$	0.183 ± 0.001 [a]	0.211 ± 0.005 [a]	0.374 ± 0.001 [a]	0.269 ± 0.002 [c]
$sCAP_{8H}$	0.142 ± 0.003 [cd]	0.161 ± 0.004 [c]	0.373 ± 0.002 [a]	0.254 ± 0.002 [d]
CAP	0.159 ± 0.002 [b]	0.162 ± 0.001 [c]	0.333 ± 0.007 [bc]	0.286 ± 0.005 [bc]
VC	0.157 ± 0.002 [b]	0.152 ± 0.002 [cd]	0.316 ± 0.001 [cd]	0.216 ± 0.001 [e]
BC	0.136 ± 0.005 [d]	0.139 ± 0.002 [e]	0.251 ± 0.001 [f]	0.167 ± 0.002 [f]

Column data marked without the same superscripts (a–f) differ significantly ($P < 0.05$).

Table 2
The HI antibody titer variation of every group (log 2).

Groups	D_7	D_{14}	D_{21}	D_{28}
$sCAP_{2L}$	5.00 ± 0.41[a]	6.50 ± 0.33[a]	6.50 ± 0.29[c]	6.50 ± 0.29[a]
$sCAP_{2H}$	4.25 ± 0.25[abc]	5.67 ± 0.33[ab]	6.25 ± 0.55[cd]	5.75 ± 0.25[b]
$sCAP_{6L}$	4.67 ± 0.33[ab]	4.25 ± 0.25[d]	7.00 ± 0.41[ab]	6.25 ± 0.29[ab]
$sCAP_{6H}$	4.75 ± 0.48[ab]	6.00 ± 0.41[a]	6.33 ± 0.33[cd]	5.75 ± 0.25[b]
$sCAP_{8L}$	4.25 ± 0.25[abc]	5.75 ± 0.25[ab]	6.25 ± 0.25[cd]	6.00 ± 0.41[ab]
$sCAP_{8H}$	4.5 ± 0.50[ab]	6.25 ± 0.25[a]	7.25 ± 0.29[a]	6.75 ± 0.25[a]
CAP	4.75 ± 0.48[ab]	5.00 ± 0.58[bc]	5.25 ± 0.25[cde]	5.00 ± 0.41[bcd]
VC	2.83 ± 0.16[c]	3.66 ± 0.21[def]	5.25 ± 0.25[de]	4.25 ± 0.48[d]
BC	2.50 ± 0.22 [d]	2.50 ± 0.29 [f]	2.16 ± 0.16[f]	3.00 ± 0.41[e]

Column data marked without the same superscripts (a–f) differ significantly ($P < 0.05$).

3. Results

3.1. The changes of peripheral lymphocyte proliferation

The changes of A_{570} values in all groups are listed in Table 1. On D_7 and D_{14}, the A_{570} values in $sCAP_{2L}$ and $sCAP_{8L}$ groups were significantly larger than those in CAP and the other groups ($P < 0.05$). On D_{21}, the A_{570} values in $sCAP_{2L}$, $sCAP_{8L}$ and $sCAP_{8H}$ groups were significantly larger than those in CAP and the other groups ($P < 0.05$). On D_{28}, the A_{570} values in all polysaccharide groups were significantly larger than those in VC and BC groups ($P < 0.05$), in $sCAP_{2L}$ and $sCAP_{6H}$ groups were significantly larger than that in CAP group ($P < 0.05$).

The highest lymphocyte proliferation rates of all polysaccharide groups are illustrated in Fig. 1. The highest lymphocyte proliferation rate in $sCAP_{2L}$ group on D_{28} was the highest (44.07%) and the following were $sCAP_{6H}$ group on D_{28} (43.87%), $sCAP_{8L}$ group on D_{14} (38.65%), $sCAP_{6L}$ group on D_{28} (34.78%), they were significantly higher than those of CAP and the other groups ($P < 0.05$).

3.2. The changes of serum antibody titer

The changes of antibody titer are listed in Table 2. On D_7, the antibody titers in all polysaccharide groups except for $sCAP_{2H}$ and $sCAP_{8L}$ group were significantly higher than that in VC group ($P < 0.05$) and in $sCAP_{2L}$ group was the highest. On D_{14}, the antibody titers in all polysaccharide groups except for $sCAP_{6L}$ group were significantly higher than that in VC group ($P < 0.05$), in $sCAP_{2L}$, $sCAP_{6H}$ and $sCAP_{8H}$ groups were significantly higher than that in CAP group ($P < 0.05$) and in $sCAP_{2L}$ group was the highest. On D_{21}, the antibody titers in $sCAP_{2L}$, $sCAP_{6L}$ and $sCAP_{8H}$ groups were significantly higher than that in VC group ($P < 0.05$), in $sCAP_{6L}$ and $sCAP_{8H}$ groups were significantly higher than that in CAP group ($P < 0.05$) and in $sCAP_{8H}$ group was the highest. On D_{28}, the antibody titers in all sCAPs groups were significantly higher than that in VC group ($P < 0.05$), in $sCAP_{2L}$ and $sCAP_{8H}$ groups were significantly higher than that in CAP group ($P < 0.05$) and in $sCAP_{8H}$ group was the highest.

3.3. The changes of IFN-γ contents

The serum IFN-γ contents in all groups are illustrated in Fig. 2. On D_{14}–D_{28}, the IFN-γ contents in all polysaccharide groups were significantly higher than those in VC and BC groups ($P < 0.05$) and in $sCAP_{2L}$ group was the highest. On D_{14}, the IFN-γ content in $sCAP_{2L}$ group was significantly higher than that in CAP group ($P < 0.05$). On D_{21}, the IFN-γ contents in $sCAP_{2L}$, $sCAP_{6L}$ and $sCAP_{8H}$ groups were significantly higher than that in CAP group ($P < 0.05$). On D_{28}, the

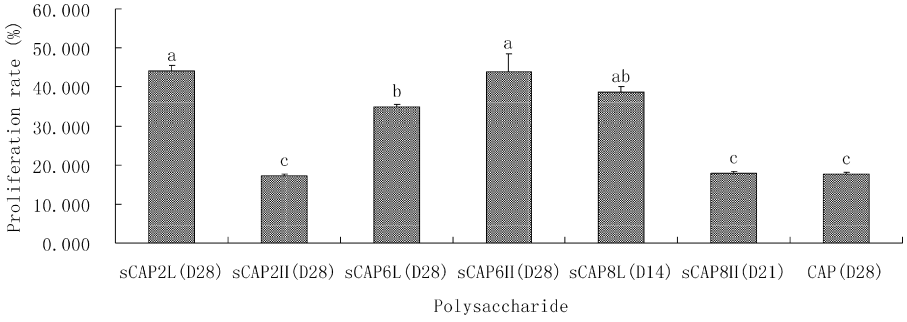

Fig. 1. The highest lymphocytes proliferation rate in every group. Bars without the same superscripts (a)–(c) differ significantly ($P < 0.05$).

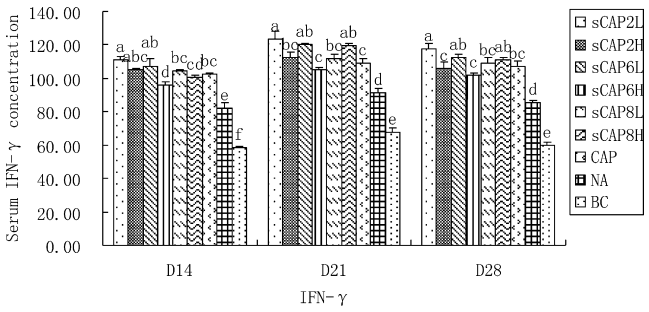

Fig. 2. The changes of IFN-γ contents of every group. Bars marked without the same superscripts (a)–(f) differ significantly ($P < 0.05$).

IFN-γ content in $sCAP_{2L}$ group was higher than that in CAP group ($P < 0.05$).

3.4. The changes of IL-6 contents

The serum IL-6 contents in all groups are illustrated in Fig. 3. On D_{14}–D_{28}, the IL-6 contents in all polysaccharide groups were significantly higher than those in VC and BC groups ($P < 0.05$) and in $sCAP_{2L}$ group was the highest. On D_{14}, the IL-6 contents in $sCAP_{2L}$, $sCAP_{6L}$, $sCAP_{8L}$ and $sCAP_{8H}$ groups were higher than that in CAP group ($P < 0.05$). On D_{21}, the IL-6 contents in $sCAP_{2L}$, $sCAP_{6L}$ and $sCAP_{8H}$ groups were higher than that in CAP group ($P < 0.05$). On D_{28}, the IL-6 contents in $sCAP_{2L}$, $sCAP_{6L}$ and $sCAP_{8H}$ groups were higher than that in CAP group ($P < 0.05$).

4. Discussion

Lymphocytes proliferation is the most direct index to reflect the cellular immune function (Ji, Peng, & Lv, 2003; Kim, Han, Oh, & Kim, 1996). It is a kind of normal protective physiological responses when the immune system is stimulated by foreign antigen therefore has very important significance for organism to resist external microbial infections. The experimental results showed that the A_{570} values in $sCAP_{2L}$ and $sCAP_{8L}$ groups on D_7 and D_{14}, in $sCAP_{2L}$, $sCAP_{8L}$ and $sCAP_{8H}$ groups on D_{21} and in all groups on D_{28} were significantly higher than that of corresponding VC group. This indicated that these polysaccharides could promote the cellular immune response of ND vaccine.

To make a comparison between selenizing and non-selenizing CAP groups, it could be seen that the A_{570} values in $sCAP_{2L}$ group on D_7–D_{28}, in $sCAP_{8L}$ on D_7–D_{21}, in $sCAP_{8H}$ group on D_{21} and in $sCAP_{6H}$ group on D_{28} were significantly higher than that of corresponding CAP group, which indicated that the cellular immune-enhancing activities of these sCAPs were significantly stronger than that of non-selenizing CAP. The comparison of the highest lymphocytes proliferation rates also displayed that lymphocytes proliferation rate in $sCAP_{2L}$ group on D_{28} was the highest, the following were

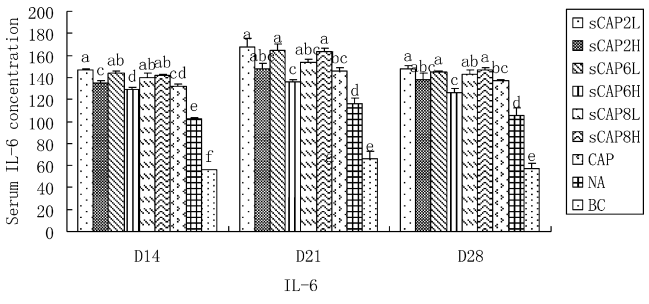

Fig. 3. The changes of IL-6 contents of every group. Bars marked without the same superscripts (a)–(f) differ significantly ($P < 0.05$).

$sCAP_{6H}$ group on D_{28}, $sCAP_{8L}$ group on D_{14} and $sCAP_{6L}$ group on D_{28}, they were significantly higher than that of non-selenizing CAP group. This confirmed that selenylation modification could significantly enhance the immune-enhancing activity of CAP and the activity of $sCAP_2$ was the strongest.

The antibody level is the marker to reflect humoral immune function (Lu, 2001; Qiu, Hu, Cui, Zhang, & Wang, 2007; Thekisoe, Mbati, & Bisschop, 2004). The experimental results showed that the serum antibody titers in all polysaccharide groups on D_7 except $sCAP_{2H}$ and $sCAP_{8L}$ groups, on D_{14} except $sCAP_{6L}$ group, on D_{21} except $sCAP_{2H}$, $sCAP_{6H}$, $sCAP_{8L}$ and CAP groups and on D_{28} except CAP group were significantly higher than that of corresponding VC group. This indicated that these polysaccharides could promote the humoral immune response of ND vaccine.

To make a comparison between selenizing and non-selenizing CAP groups, it could be seen that the serum antibody titers in $sCAP_{2L}$ group on D_{14} and D_{28}, in $sCAP_{8H}$ groups on D_{14}–D_{28}, in $sCAP_{6H}$ groups on D_{14} and in $sCAP_{6L}$ group on D_{21} were significantly higher than that of corresponding non-selenizing CAP groups, and in $sCAP_{2L}$ group on D_7–D_{14} and in $sCAP_{8H}$ group on D_{21}–D_{28} were the highest. This indicated that the humoral immune-enhancing activities of these sCAPs were significantly stronger than that of non-selenizing CAP, selenylation modification could significantly enhance the immune-enhancing activity of CAP and the activities of $sCAP_{2L}$ and CAP_{8H} were the stronger.

IFN-γ is secreted by Th1 cells, mainly promote cellular immune response and play a key role in regulation of immune system besides having broad-spectrum antiviral function (Raymond & Wilkie, 2004; Schroder, Hertzog, & Ravasi, 2004). It can connect natural and adaptive immune response, coordinate natural immune cells to recognize pathogens and induce the generation of specific immune response. The experimental results showed that at all time points, the serum IFN-γ contents in all polysaccharides groups were significantly higher than those in VC and BC groups and in $sCAP_{2L}$ group were the highest. This indicated that all polysaccharides could promote IFN-γ secretion and action of $sCAP_{2L}$ was the strongest. To make a comparison between selenizing and non-selenizing CAP groups, it could be seen that the serum IFN-γ contents in $sCAP_{2L}$ group on D_{14}–D_{28} and in $sCAP_{6L}$ and $sCAP_{8H}$ groups on D_{21} were significantly higher than that of non-selenizing CAP groups, which indicated that the efficacies of these three sCAPs were significantly stronger than that of CAP and selenylation modification could significantly enhance the immune-enhancing activity of CAP.

IL-6 is secreted by Th2 cell and plays an important role in humoral immunity. It can promote the proliferation of B cell and the production of immunoglobulin (Salgame, Abrams, & Clayberger, 1991). The experimental results showed that at all time points, the serum IL-6 contents in all polysaccharides groups were significantly higher than those in VC and BC groups, and in $sCAP_{2L}$ group were the highest. This indicated that all polysaccharides could promote IL-6 secretion and the action of $sCAP_{2L}$ was the highest. To make a comparison between selenizing and non-selenizing CAP groups, it could be seen that the serum IL-6 contents in $sCAP_{2L}$, $sCAP_{6L}$ and $sCAP_{8H}$ groups on D_{14}–D_{28} and in $sCAP_{8L}$ on D_{14} were significantly higher than that of CAP group, which indicated that the actions of these four sCAPs were significantly stronger than that of CAP and selenylation modification could significantly enhance the immune-enhancing activity of CAP.

The activity of polysaccharide depend mainly on its molecular structure, especially when certain chemical group is introduced, it often cause the changes of physicochemical nature and perspective structure of polysaccharide thus enhancing the original activity of polysaccharide or making polysaccharide generate new activity (Chaidedgumjorn et al., 2002; Ma, Guo, Wang, Hu, & Shen, 2010; Zhang & Hu, 2011). Selenizing polysaccharide is

formed with selenium and polysaccharide therefore possesses dual pharmacological activities of selenium and polysaccharide but it is not the simple addition of two kinds of activities, its activity is higher or newer as compared with selenium and polysaccharide (Cao & Zhong, 2010; Cui, Sang, & Zou, 2003; Li & Hu, 2008). The activity of selenizing polysaccharide is mainly related to its content of selenium and polysaccharide. Many research confirmed that the immune-enhancing activity of selenizing polysaccharide depended on the synergism of optimal selenium and carbohydrate content. The experimental results showed that $sCAP_{2L}$ presented the strongest activity and this may be due to CAP_2 with optimal selenium and carbohydrate content.

In conclusion, selenylation modification can significantly enhance the immune-enhancing activity of CAP and $sCAP_2$ possessed the strongest activity and would be as a component drug of new-type immunoenhancer.

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