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Isolation, purification and antitumor activity of lipopolysaccharide from cow placenta

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ABSTRACT

Lipopolysaccharide from cow placenta (LPS-CP-2) has been isolated and purified by hot phenol–water extraction, enzyme hydrolysis, chloroform–petroleum ether method, ion-exchange and gel-filtration chromatography. Also, LPS-PS-2 was evaluated for antitumor activity against Ehrlich ascites carcinoma (EAC)-bearing Swiss albino mice. LPS-PS-2 caused significant ($P < 0.05$) decrease in tumor volume, and viable cell count; and it prolonged the life span of EAC-tumor-bearing mice. Hematological profile indicates that LPS-CP-2 possessed protective action on the haemopoietic system. Further, administration of LPS-CP-2 reduced the tumor volume of both DLA and EAC cell lines in a dose-dependent way. The LPS-PS was found to be devoid of pyrogenic response in the rabbits. These results indicate that LPS-PS exhibited significant antitumor activity without pyrogenic response, suggesting its potential as antitumor agent.

1. Introduction

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is a potent activator of various immune cells including neutrophils [1], macrophages [2], and CD4 and CD8 T cells that infiltrate tumors [3], and endothelial cells through interactions with one or more Toll-like receptors [4]. LPS stimulates the secretion of cytokines [5] such as tumor necrosis factor-α (TNF-α) [6], interferon-γ (IFN-γ) [7], interleukin (IL)-1β [8], and IL-6 [9]. Also LPS could activate the kupffer cells in mice liver *in vitro*, which constitute the large group of macrophages with strong immune functions, and enhance its antitumor activity as well [10]. Because of its strong immunostimulating activity, LPS has been extensively investigated as an antitumor therapy, but its clinical application in humans has been limited by its high toxicity [11]. Lipopolysaccharides play a major role in the pathogenesis of Gram-negative infections [12]. They also have the ability to incite a vigorous inflammatory response. In humans, nanograms of LPS from some bacteria injected into the blood stream can result in all the physiological manifestations of septic shock [13,14].

However, it was reported that the use of LPS from plants [15–18], animal placenta [19] and human placenta exhibit strong antitumor activity with no or little toxicity. Human placenta is a traditional Chinese animal medicine. Many bioactive substances such as LPS, tumor suppressor and peptides, have been isolated from human placentas, which showed good antitumor activity and widely used in many diseases [20–22]. LPS from human placenta could also enhance the immune function of red cells *in vitro* [23]. All these experiments were approved by Research Ethics Committee of China, in which written informed consent was obtained in advance from all human subjects.

Many rural people in West China traditionally use dried cow placenta to cure patient suffered by "yin and yang deficiency" (asthenia universalis) in traditional Chinese medicine theory [24]. Also, many bioactive substances from cow placenta were isolated and their immunoregulating functions were estimated [25–27].

From our experience, LPS from cow placenta might have the potential to serve as effective therapeutic agent for tumor diseases, especially the inhibitors of inflammation-induced tumor. Cow placenta could easily obtained because of the rapid development of the Dairy Industry in China, which often discarded anywhere and caused a sever pollution. The aim of the present study is to isolate and examine the antitumor activity of the lipopolysaccharide

Abbreviations: Ara, arabinose; C-LPS-CP, crude LPS-CP; DLA, Dalton's lymphoma ascites; EAC, Ehrlich ascites carcinoma; Gal, galactose; LPS, lipopolysaccharide; LPS-CP-2, lipopolysaccharide from cow placenta; Man, manose; Rib, ribose; Xyl, xylose.

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isolated from cow placenta using both the ascites and solid tumor bored in mice.

2. Materials and methods

2.1. Isolation and purification of the lipopolysaccharide fractions

2.1.1. Isolation of LPS-CP

Cow placentas obtained within 30 min of normal term delivery were stripped of membranes and washed with water. Then the tissue was treated by freezing–melting (−4 and 5 °C) for three times. The tissue was cut into segments, blotted on filter paper to remove excess blood, and homogenized in the PBS buffer (0.02 M, pH 7.4) 1:2 (w/v) for 90 s at Setting 10 in a Sorvall Omni-Mixer (Sorvall Inc., USA) maintained in an ice bath. The homogenate was centrifuged at 3000 × *g* for 15 min, and the supernatant was retained. Then two aliquots of phenol–water (1:1, v/v) solution was added in the supernatant and maintained at 60 °C for 30 min, centrifuged at 6000 × *g* for 30 min [28]. The supernatant was retained and dialyzed in distilled water four times overnight at 4 °C. 2 units/ml enzyme DNase I (Keygen, China) was added in the supernatant and maintained at 45 °C, pH 5.0, for 60 min; then 2 units/ml RNase (Keygen, China) at 37 °C pH 8.0, for 4 h, and centrifuged at 6000 rpm, at 4 °C for 1 h. The thus obtained solution was dialyzed in distilled water four times overnight at 4 °C. The obtained supernatant was added with four aliquots of chloroform:petroleum ether (2:1, v/v) and maintained at 30 °C for 30 min, centrifuged at 6000 × *g* for 30 min [29]. This operation was performed for two times. The supernatant was collected. Chloroform and petroleum ether were removed by evaporation (Buchi R-210/215, Switzerland) from the supernatant. The collected LPS was washed once with 80% phenol and several times with a 1:1 diethyl ether–acetone mixture to remove any phenol. Finally, the phenol-free LPS was suspended in deionized water and collected by centrifugation at 20,000 rpm, at 10 °C for 1 h. This centrifugation step was repeated for two times. This LPS preparation is hereafter referred to as crude LPS-CP (C-LPS-CP). All water used in this study was free of pyrogen in this study.

2.1.2. DEAE-Sepharose F.F chromatography

Thirty milligrams C-LPS-CP sample above-mentioned was dissolved in 8 ml distilled water solution from above was applied to a column (35 mm × 600 mm) of DEAE-Sepharose F.F (Pharmacia, USA) equilibrated with 0.05 mM Tris/HCl buffer, pH 7.56. The column was washed with this buffer containing 0.05 M NaCl until the breakthrough peak of lipopolysaccharide had been eluted. This peak contained lipopolysaccharide and approximately 95% of the total content. The column was then eluted with a 800 ml linear gradient: 0.05–0.5 M NaCl in Tris/HCl buffer, pH 7.56. Flow rate was set at 2 ml/min and 10 ml fraction was collected. A typical elution profile is shown in Fig. 1A. The peak of lipopolysaccharide content was eluted at about 0.42 M NaCl concentration. The C-LPS-CP was combined into three fractions named LPS-CP-A, LPS-CP-B and LPS-CP-C. The pooled LPS-CP-C, the major polysaccharide-containing population, was dialyzed against distilled water at 4 °C overnight and condensed by filtration through filter paper. Thus, the obtained LPS-CP-C was further purified.

2.1.3. Bio-Gel P 10 chromatography

The LPS-CP-C sample (24 mg dissolved in 5 ml distilled water) from DEAE-Sepharose F.F column was applied to the Bio-Gel P 10 (16 mm × 750 mm, Bio-Red, USA) column and LPS-CP was washed with 0.15 NaCl. 5 ml fraction was collected. The flow rate was set at 2 ml/min. There were four fractions pooled: LPS-CP-1, LPS-CP-2, LPS-CP-3 and LPS-CP-4. The pooled LPS-CP-2 was dialyzed against distilled water at 4 °C for 36 h and lyophilized for the further study of its antitumor activity.

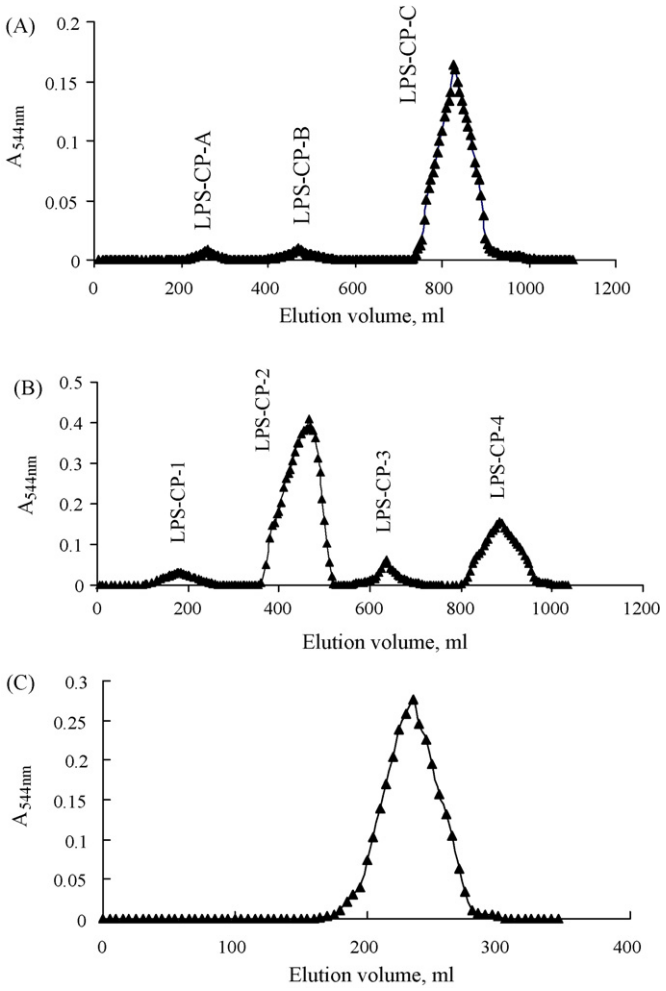

Fig. 1. Elution profiles for purification of LPS-CP-2. (A) Chromatography of crude LPS-CP on DEAE-Sepharose F.F. (B) Chromatography of LPS-CP-C on Bio-Gel P 10. (C) Chromatography of LPS-CP-2 on Sepharose CL-6B to check its chemical homogeneity.

2.1.4. Lipopolysaccharide estimation

During the purification procedure LPS-CP was determined according to the methylene blue dying method described by Chen [16,30]. The dying solution was prepared by dissolving 16 mg 1, 9-dimethylamine methylene blue (Sigma, USA) in 5 ml ethanol (80%, v/v), adding 3.33 g NaCl and being diluted to 1 l with glycine–NaOH buffer (pH 10.1, 0.057 M), then stored in amber laboratory bottle. Serial standard lipopolysaccharide (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 mg) from *Escherichia coli* 055:B5 (Sigma, USA) were respectively dissolved in 0.1 ml double-distilled water and then mixed with 3 ml dying solution, then all the mixed solutions were determined spectrophotometrically in 544 nm. Blank control was made by 0.1 ml double-distilled water mixed with 3 ml dying solution. According to these data, the standard curve of absorbance versus LPS (mg) was made. The sample content was determined using the same method stated above and the lipopolysaccharide content was read from the standard curve.

2.1.5. Homogeneity proof

10 mg sample dissolved in 0.15 M NaCl was applied to the Sepharose CL-6B (10 mm × 700 mm, Pharmacia, USA) column eluted with 0.15 M NaCl. The column was eluted with 0.15 M NaCl

with flow rate set at 0.6 ml/min. 5 ml of fraction was collected. The LPS-CP was determined by the methylene blue dying method.

2.1.6. Gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulfate (SDS) was performed on LPS-CP-2 in 7.5% acrylamide gels (85 mm × 5.5 mm), according to the method of Weber and Osborn [31]. After electrophoresis (0.5 mA/gel, 3 h), gels containing lipopolysaccharide were fixed for 2 h in 40% ethanol–5% acetic acid, overnight and then stained with silver.

2.1.7. Molecular weight estimation

Molecular weight of LPS-CP-2 was estimated by SDS-PAGE, which was performed in a continuous buffer system containing 0.1% SDS with a linear gradient of 10–20% acrylamide (Fluka, USA) cross-linked with 0.8% *N*,*N*′-methylene-bisacrylamide (Fluka, USA). Slabs were electrophoresed at a constant current of 35 mA per slab at 12 °C. Proteins included for calibration of molecular masses were 3.8, 8.4, 16, 26.6, and 36.4 kilodaltons (kDa) (Kaleidoscope, Bio-rad, USA). Slabs were fixed with 10% acetic acid–50% ethanol overnight. Visualization was accomplished with the LPS silver stain of Tsai and Frasch [32] based upon oxidation of organic groups by periodic acid.

2.1.8. Protein and sugar analysis

The protein in LPS-CP-2 was determined by the absorbance at 280 nm and the Folin-phenol reaction [33]. LPS-CP-2 was hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C in a sealed-tube for 1 h. After TFA was removed, one part of the hydrolysate was analyzed by paper chromatograph (PC) and the other part was acetylated and analyzed by gas liquid chromatography (GLC) [34].

2.2. Tumor cells

Ehrlich ascites carcinoma (EAC) cells and Dalton's lymphoma ascites (DLA) cells were obtained from Beijing BXGK Hi-Tech Development Co., Ltd., China. The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal inoculation of 2×10^6 cells/mouse. EAC and DLA cells aspirated from the peritoneal cavity of mice were washed with saline and given intraperitoneally to develop ascitic tumor to all the animals except the normal group.

2.3. Animals

Antitumor studies were carried out using male Swiss albino mice weighing 18–25 g. They were obtained from the animal house of Tianjin Pharmaceutical University. The mice were grouped and housed in polyacrylic cages (38 cm × 23 cm × 10 cm) with not more than 12 animals per cage and maintained under standard laboratory conditions (room at constant temperature 25 °C with alternating 12 h periods of light and darkness), humidity 50–60%. They were allowed access to standard dry pellet diet and water *ad libitum* before use. The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiment. The purified LPS-CP-2 was suspended in distilled water and administered by gastric perfusion to the animals with the help of an intragastric catheter to study *in vivo* antitumor activity. Pyrogen assays were performed with normal New Zealand rabbits, purchased from Beijing Laboratory Animal Research Center. This study was approved by the Committee for Ethics in Animal Research of our university and experiments were carried out in accordance with the norms of the Animal Experimentation, established by Tianjin University of Commerce.

2.4. Determination of the effect of LPS-CP-2 on ascites tumors in mice

2.4.1. Tumor growth response

The effects of LPS-CP-2 on tumor growth and host survival were estimated by evaluating tumor volume, tumor cell count and percentage increase in life span (ILS) of the tumor hosts, respectively. Four groups (6 mice/group) of Swiss albino mice were induced ascites tumor by injecting 1×10^6 cells/animals (EAC) to the peritoneal cavity. Control group was kept as control which received PBS, treated groups were given the three doses of LPS-CP-2 solution (20, 40, 60 mg/kg body weight) 24 h by gastric perfusion after the tumor transplantation for 10 consecutive days. Animals were observed for the development of ascites tumor and deaths due to tumor burden were noted. Mean survival time (MST) of each group was calculated using following equation: MST = (day of first death + day of last death)/2 [35]. The increase in life span (percent ILS) of treated group was calculated using the formula, percent ILS = $(T - C)/C \times 100$, where '*T*' and '*C*' are mean survival of treated and control mice, respectively [36]. The viable tumor cell counts (Trypan blue test) were carried out with a hemocytometer. Tryphan blue was obtained from Sigma.

2.4.2. Hematological studies

The blood was collected from the animals by retro-orbital puncture under slight anesthesia (diethyl ether) condition; and the hematological parameters such as red blood cells (RBC), white blood cells (WBC) [37], WBC differential count, and hemoglobin content [38] were determined from freely flowing tail vein blood. WBC differential count was carried out from Leishman stained blood smears [39].

2.5. Determination of the effect of LPS-CP-2 on solid tumor development

Solid tumor was induced by injecting EAC cells (1×10^6 cells/animal) subcutaneously to the right hind limbs of three groups (6 mice/group) of Swiss albino mice. Animals in treated group received LPS-CP-2 by gastric perfusion at a concentration of 20, 40, 60 mg/kg body weight, respectively, 24 h after tumor inoculation and continued for 10 days. Animals in control group were kept as untreated control, which received the vehicle. The radii of developing tumor were measured using vernier calipers at 5 days intervals for 1 month and tumor volume was calculated using the formula $V = 0.4ab^2$, where '*a*' and '*b*' represent the major and minor diameter, respectively [40]. This was compared with untreated control. The same experiment protocol was used in the solid tumor induced by DLA cells.

2.6. Rabbit pyrogen test

Experimental rabbits having average weight of 3 ± 0.2 kg were taken on the day of the study, and feed was withheld and rabbit was acclimatized to the test condition for 2 h. Anal temperatures of the rabbits were detected for three times at an interval of 30 min before performing the test. A rabbit was suitable for the test if the fluctuant range of the three detected temperatures was 0.2. The qualified rabbits were randomly divided into four groups and each group contained three animals. Temperature was monitored by means of thermometer inserted at least 10 cm in to the rectum and recorded by the calibrated thermometers. Three doses (20, 40, 60 mg/kg) of LPS-CP-2 solution (1 ml/kg) were injected into the marginal ear vein of rabbit. Each dose was administered to the three rabbits of the treated group. Temperature measurements were taken over an hour period at 50 min interval. The positive standard for rabbit pyrogen

Table 1
The effect of LPS-CP-2 treatment on the life span and tumor growth of ascites tumor-bearing mice[a]

Groups	Dose (mg/kg)	Mean survival days	Percentage of increase in life span (% ILS)	Tumor volume (ml)	Viable tumor cell count ($\times 10^7$)	Nonviable tumor cell count ($\times 10^7$)
EAC Control	–	19.0 ± 1.22*	–	3.82 ± 0.32	9.13 ± 0.24	0.53 ± 0.37
LPS-CP-2	20	23.0 ± 1.54*	21.51	3.25 ± 0.16*	8.18 ± 1.01*	0.73 ± 0.46*
	40	27.1 ± 1.31*	42.38	3.09 ± 0.26*	7.46 ± 0.87*	0.78 ± 0.25*
	60	29.2 ± 2.61*	53.46	2.73 ± 0.35*	4.36 ± 0.95*	0.85 ± 0.12*

[a] Results presented as mean ± S.E.M. ($n = 6$).
* $P < 0.05$ (ANOVA; LSD post hoc test) between tumor control and treated groups.

assay was described as following: body temperature for any one of the three rabbits in one group showed 0.6 elevation, or the total elevated body temperature for the three rabbits in one group was 1.4.

2.7. Statistical analysis

All values reported are the mean ± S.E.M. for each group. Statistical significance between groups was calculated by analysis of One Way ANOVA (Fisher LSD post hoc test). The SPSS software package was used for all analyses. Differences were considered to be significant if $P < 0.05$.

3. Results and discussion

3.1. Isolation and purification of the lipopolysaccharide fractions

Elution profiles for purification of LPS-CP were shown in Fig. 1A–C. The LPS-CP-2 was proved chemically homogeneous by gel-filtration. Molecular weight of the LPS-CP-2 estimated by SDS-PAGE was 8 kDa. The lack of absorbance at 280 nm and its negative reaction in the Folin-phenol reaction indicated that this polysaccharide contained no protein. The sugar composition of LPS-CP was determined by PC and GLC as alditol acetate derivatives. LPS-CP-2 was composed mainly of Rib, Man, Gal, Ara and Xyl in a molar ration of 1:6:8.4:10:13.

3.2. Effect of LPS-CP-2 on ascites tumors

LPS-CP-2 administration increased the life span of ascites tumor harboring mice. All the untreated mice in the EAC-tumor group died of tumor burden average 19.0 ± 1.22 days after tumor inoculation. Administration of LPS-CP-2 extract (20, 40, 60 mg/kg body weight by gastric perfusion) increased the survival of animals to 23.0 ± 1.54, 25.3 ± 1.31 and 29.3 ± 2.61 days (Table 1). Administration of LPS-CP-2 by gastric perfusion significantly increased the life span of the tumor-bearing mice compared to the control group. The percentage of increase in life span was 21.51, 42.38 and 53.46% respectively at the dose of 20, 40, 60 mg/kg (Table 1). Also, a regular rapid increase in ascites tumor volume was noted. Treatment with LPS-CP-2 decreased the ascites fluid volume, viable cell count, and increased the percentage of life span. Ascites fluid is the direct nutritional source for tumor cells and a rapid increase in ascitic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells. The reliable criteria for judging the value of any antitumor drug are the prolongation of the life span of animals. It might be concluded that LPS-CP-2 by decreasing the nutritional fluid volume and arresting the tumor growth increases the life span of EAC-bearing mice.

3.3. Effect on hematological parameters

As shown in Table 2, RBC, Hgb, lymphocyte, and eosinophils were decreased and the total WBC and neutrophils count were significantly increased in the EAC control group as compared to the normal group. Treatment with LPS-CP-2 at the three doses (20, 40, 60 mg/kg) all significantly increased the Hgb content, RBC and eosinophils and significantly decreased the total WBC and the neutrophils count to about normal levels. A significant decrease in hemoglobin and the number of RBC and a significant increase in total WBC in the tumor-bearing mice are known. Anemia is found frequently in cancer patients. The reversal of Hgb content, RBC, platelets, and differential count of WBC by the LPS-CP-2 treatment towards the values of the normal group clearly indicates that LPS-CP-2 possessed protective action on the haemopoietic system.

3.4. Effect of LPS-CP-2 on solid tumors

To investigate if the inhibitory effect of LPS-CP-2 on DLA and EAC cell lines was local or systemic, the effect of LPS-CP-2 in another experimental system, DLA-induced and EAC-induced solid tumor, were tested. Administration of LPS-CP-2 reduced the tumor volume of both DLA and EAC cell lines in a dose-dependent way. The tumor volume of animals without LPS-CP-2 treatment on the 30th day after tumor (EAC) inoculation was found to be 4.9 ml. The tumor volume was reduced to 3.0, 2.0, and 2.3 ml by LPS-CP-2 administration at concentrations of 20, 40 and 60 mg/kg body weight, respectively (Table 3). Similarly, the tumor volume of DLA inocu-

Table 2
The effect of LPS-CP-2 on hematological parameters of EAC-bearing mice on day 10 of the experiment

Groups	Dose (mg/kg)	Total WBC ($\times 10^3/mm^3$)	RBC ($\times 10^6/mm^3$)	Hgb (g/dl)	WBC differential count (%)		
					Lymphocytes	Neutrophils	Eosinophils
Normal	–	9.65 ± 0.26	10.43 ± 0.351	15.95 ± 0.525	64.84 ± 0.655	28.92 ± 1.03	4.91 ± 0.221
EAC Control	–	14.28 ± 0.63[a]	5.56 ± 1.05[a]	7.61 ± 0.26[a]	35.77 ± 0.71[a]	63.95 ± 1.37[a]	3.08 ± 0.52[a]
LPS-CP-2	20	14.28 ± 1.05*	9.25 ± 0.24*	12.14 ± 0.18*	57.44 ± 1.56*	34.26 ± 1.72*	4.06 ± 0.33*
	40	12.94 ± 0.72*	9.30 ± 0.31*	12.93 ± 0.33*	59.37 ± 1.21*	36.67 ± 1.23*	4.22 ± 0.24*
	60	10.56 ± 0.75*	10.14 ± 0.14*	14.06 ± 0.62*	62.82 ± 1.02*	39.44 ± 1.85*	4.78 ± 0.56*

Values are expressed as the mean ± S.E.M. ($n = 6$) on day 10 of the experiment.
[a] $P < 0.05$ (ANOVA; LSD post hoc test) between normal and tumor control group.
* $P < 0.05$ (ANOVA; LSD post hoc test) between tumor control and treated groups.

Table 3
The effect of LPS-CP-2 administration on solid tumor volumes induced by EAC cell in mice

Groups	Dose (mg/kg)	Tumor volume (ml)[a]					
		5 days	10 days	15 days	20 days	25 days	30 days
Control	–	0.38 ± 0.03	0.41 ± 0.02	1.2 ± 0.05	2.5 ± 0.54	3.7 ± 0.54	4.9 ± 0.82
LPS-CP-2	20	0.37 ± 0.02	0.40 ± 0.03	0.74 ± 0.08*	1.8 ± 0.41*	2.8 ± 0.37*	3.0 ± 0.46*
	40	0.38 ± 0.04	0.38 ± 0.03	0.72 ± 0.07*	1.2 ± 0.67*	2.1 ± 0.15*	2.6 ± 0.58*
	60	0.36 ± 0.01	0.38 ± 0.01*	0.71 ± 0.06*	1.1 ± 0.24*	1.6 ± 0.26*	2.3 ± 0.23*

[a] Tumor volume are mean ± S.E.M. ($n = 6$).
* $P < 0.05$ (ANOVA; LSD post hoc test).

Table 4
The effect of LPS-CP-2 administration on solid tumor volumes induced by DLA cell in mice

Groups	Dose (mg/kg)	Tumor volume (ml)[a]					
		5 days	10 days	15 days	20 days	25 days	30 days
Control	–	0.24 ± 0.03	0.56 ± 0.04	1.4 ± 0.55	3.5 ± 0.73	5.8 ± 0.94	6.9 ± 1.11
LPS-CP-2	20	0.25 ± 0.02	0.54 ± 0.08	1.2 ± 0.30*	3.2 ± 1.03*	5.3 ± 0.88*	6.0 ± 0.94*
	40	0.23 ± 0.04	0.55 ± 0.07	0.8 ± 0.14*	2.6 ± 0.93*	4.2 ± 0.97*	4.8 ± 1.07*
	60	0.22 ± 0.03	0.51 ± 0.05	0.7 ± 0.23*	1.9 ± 0.77*	3.3 ± 0.92*	3.4 ± 1.04*

[a] Tumor volume are mean ± S.E.M. ($n = 6$).
* $P < 0.05$ (ANOVA; LSD post hoc test).

Table 5
The pyrogenic effect of LPS-CP-2 in rabbits[a]

Groups	Dose (mg/kg)	Rectal temperature (°C)		
		0 h	2 h	3 h
Control	–	37.4 ± 0.02	37.3 ± 0.02	37.4 ± 0.15
LPS-CP-2	20	37.5 ± 0.01	37.6 ± 0.02	37.8 ± 0.24
	40	37.6 ± 0.24	37.5 ± 0.01	37.8 ± 0.26
	60	37.8 ± 0.05	37.6 ± 0.02	37.5 ± 0.03

[a] Results presented as mean ± S.E.M. ($n = 3$).

lated animals on the 30th day after tumor inoculation was found to be 6.9 ml which was reduced to 6.0, 4.8 and 3.4 ml and the percent reduction in tumor volume was found to be 13.0, 30.4 and 50.7% in the group of animals treated with LPS-CP-2 (Table 4). The solid tumor was inhibited by treatment with LPS-CP-2, suggesting that the inhibitory effect is related not only to a local cytotoxic effect but also with the systemic effect of LPS-CP-2.

3.5. Pyrogenic response

The observations for rabbit pyrogen test were tabulated in Table 5. LPS-CP-2 exhibited no pyrogenicity at all doses in normal New Zealand rabbits. LPS-CP-2 could not produced fevers in rabbits and be considered safe at the doses in this experiment.

All these results suggested the antitumor nature of the LPS-CP-2 and among the three doses, the antitumor activity was dose-related with the lower dose of 20 mg/kg body weight was found to be sufficiently effective.

4. Conclusion

In this study, LPS-CP-2 has been isolated and examined its antitumor activity. The results showed that LPS-CP-2 was effective in prolonged the life span of EAC-bearing mice. Also, hematological profile indicates that LPS-CP-2 possessed protective action on the haemopoietic system. Further, administration of LPS-CP-2 reduced the tumor volume of both DLA and EAC cell lines in a dose-dependent way. The LPS-PS was found to be devoid of pyrogenic response in the rabbits. These results indicate that LPS-PS-2 exhibited significant antitumor activity in EAC-bearing mice without pyrogenic response, suggesting its potential as antitumor agent. Further research work to establish the antitumor mechanism of action of LPS-PS-2 is in progress in our laboratory.

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