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# Blood Component Change in Rat by Lipopolysaccharide and Cell Wall Protein-A from *Vibrio vulnificus*, *E. coli*, and *S. typhimurium*

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**Abstract** Lipopolysaccharide (LPS) and cell wall protein-A (CWP-A) were extracted from the cell wall of *Vibrio vulnificus*, *Escherichia coli* and *Salmonella typhimurium*. LPSs and CWP-As were injected into rat and the changes of the following blood components were examined. The change of the number of white blood cell (WBC), red blood cell (RBC), platelet (PLT), reticulocyte (RETI) and partial thromboplastin time (PTT), blood urea nitrogen (BUN) and blood glucose in rat blood and interferon (IFN) activity change by LPS and CWP-A were measured. WBC, RETI, PTT, and BUN were increased and RBC and blood glucose were increased slightly, but PLT was decreased.

**Key words**: *blood component change, LPS, CWP-A*

## Introduction

Parenteral administration of endotoxin may greatly alter an animal's response to a subsequent challenge with infectious agents. For instance, when given shortly before an infectious challenge, resistance to infection may be markedly diminished. Whether or not this phenonmenon is relevant to the pathogenesis of naturally acquired gram-negative bacterial infection, the possible role of endotoxin has been a subject of considerable interest. The problem of the role of endotoxin in infection has been a difficult one to approach experimentally. However, one interesting area of investigation has evolved about the possibility that endotoxin may be responsible for the lethal outcome of mouse typhoid. The parenteral administration of a rather small dose of endotoxin renders animals, tolerant to a lethal endotoxin dose and also increases their natural resistance.

Lipopolysaccharide (LPS, endotoxin) forms an unique component of the outer membrane of gram-negative bacteria. LPS serves a crucial role in the barrier function of the outer membrane by virtue of its complex lipid moiety anchoring it in the membrane and specific interactions of anionic groups in the carbohydrate portion with divalent ions such as $Mg^{2+}$ and $Ca^{2+}$. LPSs are known to consist of a phosphorylated and extensively acylated glucosamine disaccharide, "lipid A", to which is linked a core oligosaccharide and a strain-specific polysaccharide moiety [1-3]. LPS possesses a wide range of biological activiites [2], most of which reside in the lipid A portion. There are many specific proteins in the outer membrane of the gram-negative bacteria. Among these, cell wall protein-A (CWP-A) is composed as major component ; in the case of E. coli K2, 39.9% and V. cholera NAG 4715, 60.6%. CWP-A may be an antigen in the specific immunological reaction and has a mitogenic activity for B lymphocyte [4]. In spite of above facts, reports about the biological activity of CWP-A are very few.

In this paper *V. vulnificus*, *E. coli* and *S. typhimurium* LPSs and CWP-As were extracted, and blood component changes in rat by LPSs and CWP-As were tested.

## Materials and Methods

### Lipopolysaccharide (LPS)

LPSs from *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, and *Vibrio vulnificus* P-1 were extracted by a modification method of the hot phenol-water procedure [5]. The bacterial cells were cultured in tryptic soy broth at 37°C for 24h. The cells were collected from the growth medium by centrifugation and were suspended in distilled water, sonicated, and centrifuged. The supernatant was centrifuged and the resulting pellet was suspended in 50 ml of distilled water and LPS was extracted with 50 ml of 90% phenol. The mixture was stirred at 65-68℃ for 20 min, cooled in ice bath, and centrifuged. Hexadecyl trimethyl ammonium bromide was added to the supernatant and centrifuged. The upper aqueous phase containing LPS was dialyzed against distilled water and freeze-dried.

### Cell Wall Protein A

Cell wall protein As from gram-negative bacteria were extracted by using Osborn's method [6]. The bacterial cells

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were washed with distilled water, suspended in 0.75M sucrose-10mM tris-phosphate buffer (pH 7.8) and added 100 μg of lysozyme per ml of suspension. After addition of two fold volumes of 1.5mM EDTA-$2Na^+$, cell membrane was obtained by centrifugation. Cell membrane was suspended in 2% triton-X-100-10mM tris-phosphate-EDTA buffer (pH 7.5) and centrifuged. This fraction was applied to DEAE-Sepharose column chromatography (3×65cm) and eluted with 0.1% triton-X-100-10mM tris-phosphate buffer (pH 7.5) using NaCl gradient. 10% Sodium cholate was added to the major fraction and applied to Sepharose S-200 column chromatography (1.5×50 cm). After removal of triton X-100, the main fraction was freeze-dried.

### Biological Responses by LPS and CWP-A

LPS and cell wall protein A dissolved in phosphate buffered saline (PBS, pH 7.4) 0.1ml were injected into rats to compare biological responses of those of *V. vulnificus, E. coli,* and *S. typhimurium*. After 0, 6, 12, and 18 h, blood was obtained by heart puncture with a 15 gauge needle. After heart puncture, blood was drawn directly into sterile pyrogen-free test tube. The number of white blood cell (WBC), red blood cell (RBC), platelet (PLT), reticulocyte (RETI), and partial thromboplastin time (PTT), blood urea nitrogen (BUN), and blood sugar in rat blood by using Coulter STIV, brilliant cresyl blue, Gilford Impac 400E, and Cobas Mira were measured.

## Results and Discussion

### Extraction of LPSs and CWP-As

The final yields of LPSs and CWP-As from *V. vulnificus*, *E. coli*, and *S. typhimurium* were shown in Table 1.

### WBC Change by LPS and CWP-A

WBC number of the control group (normal blood) was 8,500 ±26.3/$mm^3$. WBC was increased in almost experimental groups except 6h and 12h after challenge group of *S. typhimurium* LPS and CWP-A (Table 2). The highest WBC was 6h after challenge group of *V. vulnificus* LPS and CWP-A (271 and 272% increase). This increase was thought to be for the removal of foreign toxic substance, LPS and CWP-A.

### RBC Change by LPS and CWP-A

RBC number of the control group was $7.65 \pm 0.25 \times 10^6$ / $mm^3$ and RBC change by LPS and CWP-A was small (Table 3).

### PLT Change by LPS and CWP-A

PLT was very sensitive to LPS and easily destroyed by LPS. Table 4 has shown that PLT was decreased very highly, especially 12h after challenge group of three bacteria's LPS and CWP-A (2, 2, and 1.6% increase).

### RETI Change by LPS and CWP-A

**Table 1.** The final yields of LPS and CWP-A

| bacteria | LPS (mg) | CWP-A (mg) |
|---|---|---|
| *V. vulnificus* | 13 | 32 |
| *E. coli* | 17 | 21 |
| *S. typhimurium* | 15 | 23 |

**Table 2.** WBC change by LPS and CWP-A.

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 231±12.6 | 159±16.0 | 80±21.4 |
| | CWP-A | 235±10.8 | 144± 9.8 | 76±20.5 |
| | LPS+CWP-A | 218±13.2 | 134±27.2 | 39±17.3 |
| 12h | LPS | 129±20.8 | 146±23.0 | 69±23.5 |
| | CWP-A | 148±15.0 | 128±24.5 | 73±26.7 |
| | LPS+CWP-A | 133±26.0 | 171±29.5 | 66±20.9 |
| 18h | LPS | 159±16.0 | 132±18.5 | 178±18.4 |
| | CWP-A | 143±21.0 | 135±15.8 | 211±24.6 |
| | LPS+CWP-A | 132±17.4 | 115±13.4 | 147±20.5 |

control group : $85.0 \pm 26.3 \times 10^2/mm^3$

**Table 3.** RBC change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhumurium* |
|---|---|---|---|---|
| 6h | LPS | 7.81±0.34 | 7.81±0.52 | 7.25±0.19 |
| | CWP-A | 7.66±0.45 | 7.66±0.35 | 7.26±0.52 |
| | LPS+CWP-A | 7.43±0.27 | 7.63±0.59 | 7.46±0.23 |
| 12h | LPS | 7.36±0.21 | 7.42±0.21 | 7.01±0.45 |
| | CWP-A | 7.23±0.13 | 7.36±0.62 | 6.78±0.29 |
| | LPS+CWP-A | 7.12±0.28 | 7.12±0.27 | 7.19±0.16 |
| 18h | LPS | 7.41±0.35 | 7.37±0.19 | 7.42±0.19 |
| | CWP-A | 7.83±0.58 | 7.83±0.55 | 7.38±0.49 |
| | LPS+CWP-A | 6.95±0.51 | 6.95±0.69 | 7.46±0.51 |

control group : $7.65 \pm 0.25 \times 10^6/mm^3$

**Table 4.** PLT change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 4.40±0.78 | 4.30±0.78 | 4.50±0.78 |
| | CWP-A | 7.68±0.34 | 7.58±0.34 | 7.72±0.34 |
| | LPS+CWP-A | 1.20±0.58 | 1.10±0.58 | 1.18±0.58 |
| 12h | LPS | 1.38±0.38 | 1.28±0.38 | 1.28±0.38 |
| | CWP-A | 3.05±0.14 | 3.15±0.14 | 3.15±0.14 |
| | LPS+CWP-A | 1.61±1.31 | 1.70±1.31 | 1.60±1.31 |
| 18h | LPS | 4.18±0.45 | 4.28±0.45 | 4.08±0.45 |
| | CWP-A | 6.35±0.74 | 6.25±0.74 | 6.05±0.74 |
| | LPS+CWP-A | 9.38±1.87 | 9.48±1.87 | 9.18±1.87 |

control group : $8.61 \pm 0.56 \times 10^4/mm^3$

RETI was formed in bone marrow, was released 4 days before RBC was fully grown and was existed in 0.5 - 1.5% of normal animal's body. RETI of the control group was

1.2±0.4% of RBC. RETI was increased more than two fold by *V. vulnificus* LPS, CWP-A, and LPS+CWP-A, and the highest RETI was 6h after challenge group of *S. typhimurium* LPS + CWP-A (Table 5).

## PTT Change by LPS and CWP-A

PTT was the test to know the deficiency of coagulation factor. PTT value of the control group was 18.0±3.6sec. PTT was increased as the reaction was proceeded and the kinds of bacterial strains did not affect to the PTT change (Table 6). The elongation of PTT was supposed to be related to the PLT breakage.

## BUN Change by LPS and CWP-A

BUN concentration was measured in order to know the effect of LPS and CWP-A on the kidney function and its results were presented in Table 7. The highest BUN was the 6h after challenge group of *V. vulnificus* LPS.

## Blood Glucose Change by LPS and CWP-A

It was reported that the glycogen concentration was reduced in liver and muscle by LPS, the activity of pyruvate kinase was changed in liver and the activities of glucokinase and phosphofructo kinase in the sugar catabolism were decreased. In this experiment the blood sugar change by LPS and CWP-A was shown in Table 8. Blood sugar concentration was similar or slightly elevated value than the control group. The highest blood glucose was the 18h after challenge group of *V. vulnificus* LPS.

**Table 5.** RETI change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 2.5±0.3 | 1.7±0.5 | 4.1±0.7 |
| | CWP-A | 2.6±0.6 | 2.1±0.6 | 2.0±0.3 |
| | LPS+CWP-A | 2.7±0.4 | 3.9±0.7 | 5.1±0.5 |
| 12h | LPS | 3.2±0.7 | 2.6±0.6 | 2.8±0.5 |
| | CWP-A | 3.6±0.6 | 2.6±0.4 | 1.7±0.4 |
| | LPS+CWP-A | 4.0±0.6 | 2.4±0.3 | 1.9±0.5 |
| 18h | LPS | 2.9±0.3 | 4.2±0.3 | 1.2±0.3 |
| | CWP-A | 2.2±0.4 | 3.1±0.5 | 1.0±0.4 |
| | LPS+CWP-A | 2.6±0.3 | 4.7±0.6 | 1.4±0.5 |

control group : 1.2±0.4 %

**Table 6.** PTT change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 22.5±2.3 | 12.6±1.3 | 22.4±2.3 |
| | CWP-A | 13.7±2.6 | 13.4±1.4 | 24.9±2.6 |
| | LPS+CWP-A | 14.7±3.5 | 18.4±1.9 | 15.2±1.6 |
| 12h | LPS | 28.1±3.5 | 24.5±2.6 | 26.9±2.7 |
| | CWP-A | 33.5±3.4 | 23.6±2.5 | 30.4±3.1 |
| | LPS+CWP-A | 21.3±2.5 | 20.8±2.1 | 24.2±2.5 |
| 18h | LPS | 29.7±3.1 | 34.6±3.6 | 39.8±4.0 |
| | CWP-A | 30.5±3.1 | 33.7±3.4 | 33.5±3.5 |
| | LPS+CWP-A | 24.7±2.6 | 24.2±2.5 | 25.1±2.6 |

control group : 18.0±3.6 sec

**Table 7.** BUN change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 56.5±5.2 | 34.2±3.0 | 38.7±4.0 |
| | CWP-A | 37.6±3.2 | 29.1±2.0 | 20.2±1.5 |
| | LPS+CWP-A | 29.0±3.0 | 40.4±6.0 | 15.8±2.4 |
| 12h | LPS | 34.6±4.3 | 30.1±2.0 | 25.1±1.3 |
| | CWP-A | 29.5±3.2 | 25.6±3.0 | 34.1±3.4 |
| | LPS+CWP-A | 26.6±2.5 | 28.8±3.0 | 29.8±1.9 |
| 18h | LPS | 28.4±2.12 | 50.0±2.3 | 23.1±2.1 |
| | CWP-A | 25.6±1.3 | 29.0±2.7 | 20.6±2.4 |
| | LPS+CWP-A | 25.9±3.5 | 23.2±3.2 | 27.4±2.6 |

control group : 21.0±2.0mg/dl

**Table 8.** Blood glucose change by LPS and CWP-A

| collection time | bacteria | *V. vulnificus* | *E. coli* | *S. typhimurium* |
|---|---|---|---|---|
| 6h | LPS | 86.6±8.5 | 86± 5.0 | 83± 5.0 |
| | CWP-A | 113.0±5.0 | 113±10.4 | 92± 4.0 |
| | LPS+CWP-A | 79.0±3.0 | 83± 5.0 | 119± 8.4 |
| 12h | LPS | 116.0±2.0 | 110± 4.0 | 119± 7.3 |
| | CWP-A | 118.0±5.0 | 121± 5.3 | 129± 5.8 |
| | LPS+CWP-A | 92.0±7.4 | 105± 3.0 | 128± 6.2 |
| 18h | LPS | 136.0±9.3 | 92± 3.0 | 135±10.2 |
| | CWP-A | 114.0±5.0 | 97± 5.8 | 131± 4.3 |
| | LPS+CWP-A | 124.0±3.4 | 111± 5.0 | 129± 5.4 |

control group : 98±16.2 mg/dl

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