Effect of *Panax notoginseng* saponins on lipopolysaccharide-induced adhesion of leukocytes in rat mesenteric venules

Kai Sun a,b, Chuan-She Wang a,c,∗, Jun Guo a, Yu-Ying Liu a, Fang Wang a, Lian-Yi Liu a, Ji-Guo He b, Jing-Yu Fana and Jing-Yan Han a,∗

a Tasly Microcirculation Research Center, Peking University Health Science Center, Beijing 100083, China
b College of Food Science and Nutritional Engng, China Agricultural University, Beijing 100083, China
c Department of Integrated Traditional Chinese and Western Medicine, School of Basic Medical Science, Peking University Health Science Center, Beijing 100083, China

Abstract. *Panax notoginseng* is the root of the Chinese traditional herb, *Panax notoginseng* (Burk) F.H. Chen. This study was aimed to investigate the inhibitory effect of *Panax notoginseng* saponins (PNS) on the leukocyte adhesion and the expression of adhesion molecules in rat mesentery venules. Male Sprague-Dawley rats were anesthetized with urethane. These were divided into control, LPS (perfused with lipopolysaccharide), and PNS group (perfused with PNS). The mesenteric microcirculation was observed under a videomicroscope. The number of adherent leukocytes, which attached to the vascular wall during more than 10 seconds, was counted along single venules (30–50 µm in diameter, 200 µm in length). The expression of adhesion molecules was examined using flow-cytometry in blood which was taken from the abdominal aorta and incubated with FITC-labeled CD11b (or CD18) antibodies. The results showed that different changes in the leukocyte adhesion and the expression of adhesion molecules among three groups. In LPS group, the leukocyte adhesion increased significantly after 20 minutes during the observation time, while it was reduced markedly in PNS group. The expression of CD11b and CD18 on the neutrophils was induced in LPS group, while it was reduced significantly in PNS group. It was suggested that PNS could reduce leukocyte adhesion in venules under the inhibitory effect on the expression of adhesion molecules (CD11b and CD18) on neutrophils.

Keywords: *Panax notoginseng* saponins (PNS), leukocyte–endothelium interaction, leukocyte adhesion molecule, lipopolysaccharide (LPS)

1. Introduction

Neutrophils express CD11b and CD18 in response to bacterial infection, ischemia and other pathological factors. These molecules enable leukocyte to bind to endothelial adhesion molecules such as intercellular adhesion molecules-1 (ICAM-1, CD54), leading to a production of oxygen radicals, which exaggerate microvascular dysfunction and tissue injury [1–3]. The inhibition of the adhesion between leukocyte and endothelial cells is one of the crucial steps in the improvement of microcirculatory disturbance which is induced by inflammation, ischemia–reperfusion (I/R), organic transplantation and thermal injury [4–7].

∗Corresponding author. E-mail: chuanshe@bimu.edu.cn (C.W.); kan@chuigaku.co.jp (J.H.).

1386-0291/06/$17.00 © 2006 – IOS Press and the authors. All rights reserved
Panax notoginseng is the roots of the Chinese traditional herb *Panax notoginseng* (Burk) F.H. Chen. *Panax notoginseng* saponins (PNS) consist of various saponins, such as notoginsenoside R1, ginsenoside Rb1, ginsenoside Rg1, which are the main effective components of the *Panax notoginseng* [8]. In China, oral and injectable Chinese medicines which use PNS as a major ingredient have been widely accepted in the therapy for the microcirculatory disturbance related diseases such as vascular disease, cerebral infarction, hepatic dysfunction. In our previous studies, cardiotonic pills (CP) which used PNS as a major ingredient could attenuate the I/R-induced adhesion of leukocyte to the venular wall in rat [9]. PNS could also inhibit the adhesion of leukocytes to hepatic sinusoid after I/R in the rat fed ethanol chronically [10]. However, little is known about whether PNS have the inhibition effect on the adhesion of leukocyte to the venular wall and the expression of adhesion molecules CD11b and CD18 of neutrophils.

It has been known that intravenous infusion of Lipopolysaccharide (LPS) could induce the adhesion of leukocytes to the venular wall. Since the infusion of LPS does not interface macrocirculation, it has been widely used as a model to investigate the mechanism of leukocyte adhesion and the effect of medicines on this process [11,12]. In the present paper, we investigated the inhibition effect of PNS on adhesion of leukocytes to the venular wall *in vivo*, using this model. Moreover, we investigated the influence of PNS on the expression of CD11b and CD18 of neutrophils using flow cytometry *in vitro*.

2. Materials and methods

2.1. Preparation of LPS and PNS solution

The LPS solution (2 mg/ml) was prepared by dissolving in saline solution. The PNS extract was received from Tianjin Talsy group (Tianjin, China). The PNS solution (5 mg/ml) was prepared by dissolving in saline solution [9].

2.2. Animal preparation

Male Sprague-Dawley (SD) rats weighing 200–250 g were provided by the Animal Center of Peking University Health Science Center. The SD rats were fasted during 12 hours before the experiment, but were allowed free access to water. They were anesthetized with urethane solution (1.25 mg/kg.bw, i.m.).

The rats (*n* = 18) were divided into three groups: control (*n* = 6), LPS (*n* = 6) and LPS+PNS group (*n* = 6). In the control group, the left jugular vein was cannulated for infusion of saline solution during 60 minutes. In the LPS group, the LPS solution was infused continuously via a catheter in the left femoral vein (2 mg/kg/hr) [12]. In the LPS group, 20 minutes before the LPS infusion, the PNS solution was infused continuously from the left jugular vein (5 mg/kg/hr) until the end of the observation.

2.3. Intravital observation of mesenteric microcirculation

In each rat, the abdomen was opened via a midline incision (20–30 mm long). The ileocecal portion of the mesentery (10–15 cm caudal) was gently drawn out, exteriorized and mounted on a transparent plastic stage. The mesentery was kept warm and moist by continuous superfusion with saline solution at 37°C. The mesenteric microcirculation was observed under an inverted microscope (DM-IRB, Leica, Germany) using a transilluminator. A color video camera (Jk-TU53H, Toshiba, Japan) was mounted on the microscope, and the image was projected onto a monitor (J2118A, TCL, Korea). The images were recorded using a DVD videocassette recorder (DVR-R25, Malata, China).
2.4. Image analysis

Single and unbranched venules with diameter ranging from 30–50 µm and length approximately 200 µm were chosen for the present study [13].

2.4.1. Venular diameter

Based on the recorded videoimages, venular diameters were measured using Image-Pro Plus 5.0 software (Media Cybernetic, USA). The diameter was determined by means of three time measurements at one site every ten minutes.

2.4.2. Adherent leukocytes

To examine leukocyte adhesion in venules, dynamic behavior of leukocytes was reviewed by replaying the recorded videoimages. In the present analysis, adherent leukocytes were defined as cells that attached to the same site during more than 10 seconds. The number of adherent leukocytes was counted along venules (30–50 µm in diameter, 200 µm in length) which were selected randomly from the recorded images [13].

2.5. In vitro experiment for expression of adhesion molecules

The expression of CD11b and CD18 was examined in vitro in another group of rats (n = 6). Blood was taken from the abdominal aorta, and anticoagulated with heparin (20 u/ml blood). The blood taken from each rat was divided into four groups as follows: control (n = 6), LPS (n = 6), PNS1+LPS (n = 6) in which PNS concentration is low (0.2 mg/ml), and PNSII+LPS group (n = 6) in which PNS concentration is high (2.0 mg/ml).

The blood was incubated with FITC-labeled CD11b (or CD18) antibodies (BD biosciences Pharmin-gen, USA) during 20 minutes at room temperature, and then lysed with haemolysin (BD biosciences Immunocytometer Systems, USA). The cells were washed twice with phosphate buffer solution (PBS; pH = 7.4). Flow cytometry (FACS Calibur, B.D.Co, USA) was used to access the mean fluorescence intensity. Five thousand neutrophils were acquired for each sample, and the mean fluorescence intensities were evaluated [14].

2.6. Statistical analysis

All values were reported as mean ± SE. Statistical significance was calculated using ANOVA and F-test for comparison of data among different treatment groups. A value of P < 0.05 was designed as significant.

3. Results

3.1. The venular diameters

The venular diameter was measured in one vessel in each rat. The diameters (mean ± SE, n = 6) were 45.9 ± 3.2, 48.8 ± 2.3 µm and 41.0 ± 4.1 µm in the control, LPS and LPS+PNS group, respectively. There appeared no significant difference among the three groups during the period of the observation time.
3.2. The number of leukocytes adherent to the venular wall

Figure 1 shows the time course of the leukocyte adhesion in rat mesenteric venules in control, LPS and PNS+LPS groups. The number of leukocytes adherent to the venular wall was 1.7 ± 0.7 cells/vessel at the end of observation in the control group. It started to increase at 20 min after LPS infusion, increasing up to 60 min during LPS infusion. In the PNS group, it was reduced significantly, compared to that in LPS group. It is to be noted that PNS caused significantly attenuation in leukocyte adhesion in venules.

3.3. The expression of adhesion molecules (CD11b and CD18)

Figures 2 and 3 show fluorescent intensities of CD11b and CD18 measured in the control, LPS, PNSI+LPS, and PNSII+LPS groups, respectively.

Compared with the control, the expression of CD11b of neutrophils increased significantly after the incubation with LPS. The LPS induced-expression of CD11b was significantly reduced by addition of PNS at concentration of 2.0 mg/ml (Fig. 2) with no alteration in the expression of CD11b at the PNS concentration of 0.2 mg/ml.
The similar result was observed for the expression of adhesion molecules CD18. LPS markedly up-regulated the expression of CD18 of the neutrophils in comparison with the control. PNS did not inhibit the expression of CD18 at the concentration of 0.2 mg/ml, while PNS significantly down-regulated the expression of CD18 when the concentration increased to 2.0 mg/ml (Fig. 3).

4. Discussion

The present in vivo experiment demonstrated that PNS attenuated the number of adherent leukocyte to venule wall in rat mesentery induced by LPS. Moreover, the in vitro experiment demonstrated that PNS also inhibited the LPS induced-expression of CD11b and CD18 of neutrophils.

Our continuous intravenous infusion of LPS at low concentration (2 mg/ml) could activate the leukocyte, elicit adhesion of leukocyte to venule wall without interfering with circulation [12,14]. LPS up-regulated the expression of adhesion molecules CD11b and CD18, which resulted in an injury to endothelial cells directly and enhancement of the expression of ICAM-1 of endothelial cells [15–17]. The binding between CD11b and CD18 of neutrophils and ICAM-1 of the endothelial cells promote adhesion of the leukocyte to endothelial cells [18]. Our study demonstrated that at the early stage of the LPS continuous infusion, leukocytes bound to the venular wall with no change in venule diameter. It was also observed that the expression of CD11b and CD18 of neutrophils was up-regulated by LPS in vitro. These results suggested that the major cause for this process might be the increase in the expression of CD11b and CD18 of neutrophils. Continuous infusion of PNS significantly reduced the adhesion of leukocyte to venule wall in vivo, PNS also inhibited the LPS-induced expression of CD11b and CD18 of neutrophils in vitro. These results suggested that PNS reduced the adhesion of leukocyte to venule wall possibly via inhibition of the expression of CD11b and CD18 of neutrophils.

It has been reported that endothelial dysfunction has also an important role in the endotoxin shock [19]. When activated, endothelium produces pro-inflammatory substance such as platelet-activating factor (PAF), oxygen free radicals and expression of adhesion molecules. In particular, the expressed endothelial receptor ICAM-1 can then bind with an upregulated expression of CD11/CD18 on the neutrophils and aggravate microcirculatory disturbance [20]. Our study suggested that PNS could improve the microcirculatory condition via attenuating the adherent leukocyte to the endothelial cells. Since this study didn’t directly observe the injury state of vessel endothelial cells, the expression of ICAM-1 on endothelial cells and the peroxide emergency on the vessel wall, we can’t predict whether PNS have the protect effect for the vessel endothelial cells and whether PNS could directly affect the peroxide
emergency. The mechanism of the effect of PNS on reducing the LPS-induced adhesion of leukocytes to venule wall in rat mesentery needs a further research.

Acknowledgement

This study was supported financially by Tianjin Tasly group, Tianjin, China.

References
