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Effect of propofol on generation of inflammatory mediator of monocytes

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ABSTRACT

Objective: To evaluate the effect of propofol with different concentrations on the expression of inflammatory mediators of interleukin and tumor-necrosis factor-α (TNF-α) by stimulating the mouse primary monocytes and human monocytic cell line with lipopolysaccharide (LPS) and also discuss the effect of propofol on the secretion of inflammatory mediator and its possible molecular mechanism.

Methods: The mononuclear cells of mouse spleen were separated and then purified to obtain the primary monocytes. The dose-effect relationship of production of pro-inflammatory cytokines by monocytes which were stimulated by LPS, namely the monocytes were stimulated by the dose of 0–500 ng/mL for 24 h. ELISA was employed to detect the concentration of IL-6, IL-8 and TNF-α. The effect of propofol on the secretion of above pro-inflammatory cytokines by the monocytes was observed. Cells were divided into the control group, the 0.1% DMSO group, the LPS group and the treatment group with LPS + different dose of propofol (propofol 1–100 μg/mL). ELISA was employed to detect the concentration of IL-6, IL-8 and TNF-α. The change in the expression of important signaling molecules in Toll-like receptor and NF-κB signaling pathway was detected after THP-1 cells were treated with propofol.

Results: The concentration of TNF-α was (3 863 ± 153) pg/mL after 12 h of stimulation by LPS and then its concentration was decreased gradually. But the concentration of IL-6 and IL-8 was relatively high after 24 h of stimulation by LPS, (5 627 ± 330) pg/mL and (1 626 ± 200) pg/mL, respectively. The propofol could inhibit the expression of IL-6, IL-8 and TNF-α caused by LPS. After the intervention treatment of 50 μg/mL propofol, the concentration of IL-6, IL-8 and TNF-α was significantly decreased ($P < 0.01$).

Conclusions: The propofol can inhibit the expression of TLR-4 and NF-κB to inhibit the activation of p38 and the expression of pro-inflammatory cytokines.

1. Introduction

The propofol, namely 2,6-diisopropylphenol, has the similar structure with α-tocopherol and butylated hydroxyanisole as some kinds of rapid and short-acting anesthetic drug. It has been widely applied in the clinical anesthesia and ICU because of the rapid onset, short lasting duration, rapid awaking and fewer side effects [1,2]. In addition to the general anesthesia and sedation, the propofol can also be used to lower the blood pressure and inhibit the inflammatory response in the clinical practice. According to previous researches, the anti-inflammation of propofol involves many aspects and many signaling pathways. The inhibition against the generation of inflammatory mediator and chemotactic factors is the key feature of its anti-inflammation [3,4]. The lipopolysaccharide (LPS) is the main chemical component of endotoxin and the promoter of inflammatory response. As the main effecter cell of LPS, the monocyte can release many pro-inflammatory cytokines after being stimulated by LPS, including IL-6, IL-8 and TNF-α. Therefore, the monocyte is a key link in the inflammatory response [5–7].

The objective of this study is to study the molecular mechanism of inhibitive effect of propofol on inflammatory mediator of monocytes. The mouse primary monocytes and human monocytic cell line were chosen as the research subjects. By exploring the stimulation dose and acting time of LPS, the

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inflammatory environment at the cellular level was simulated. Cells were treated with propofol under the stimulation of LPS. ELISA was employed to detect the expression of pro-inflammatory cytokines of IL-6, IL-8 and TNF-α and then discuss the dynamic effect of propofol on the generation of inflammatory mediator of monocytes under the stimulation of LPS. LPS was bound with LPS binding protein on the monocytes to activate the downstream signaling pathway. Real-time PCR was employed to detect and analyze the expression of node molecules in the related signaling pathways, in order to discuss the possible anti-inflammation molecular mechanism of propofol.

2. Materials and methods

2.1. Materials and reagents

The human monocyte THP-1 was purchased from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences. A total of 15 specific pathogen free Balb/c mice, male or female, with the weight of (15 ± 5) g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. Mice were fed with the standard pellet diet in the standard animal cage, with 5 mice in each cage. During the experiment, mice could eat and drink freely. The feeding room had the good ventilation and natural lighting day and night. The room temperature was maintained at 18–25 °C.

The cell strainer was purchased from BD Falcon (the USA)-REF352350; the mouse percoll from Dakewe Biotech Company; RPMI-1640 culture medium from Hyclone (the USA); the fetal bovine serum from GIBCO (the USA); the propofol from SIGMA (the USA)-D126608; the lipopolysaccharide from SIGMA (the USA)-L2630; IL-6 ELISA kit from Abcam (the USA): ab100713 (mouse)/ab178013 (human); IL-8 ELISA kit from Abcam (the USA): ab46103 (mouse)/ab185986 (human); TNF-α ELISA kit from Abcam (the USA): ab100747 (mouse)/ab181421 (human); total RNA extraction kit from Ambion (the USA)-12183-555; reverse transcription kit from Applied Biosystems (the USA)-4366597; Real-time PCR fluorescent quantitative kit from Bio-Rad (the USA)-172-5264; Anti-p38/Anti-p38 (phospho T180) monoclonal antibody from Abcam (the USA)-ab7952/178867; horseradish peroxidase (HRP) labeled secondary antibody from Beijing Zhongshan Jinqiao Biotechnology.

The auto ELISA detector was Shanghai Utrao-SM600; RNA quantitative analyzer: Qubit Fluorometer; optical microscope: Olympus BX53; CO_2 incubator: Thermo Scientific Series 8000; Fluorescent quantitative PCR detection system: Bio-Rad-CFX96 Touch.

2.2. Methods

2.2.1. Separation of primary cells and cell culture and treatment

After 7 d of adaptive feeding, the mononuclear cells were separated from the spleen of Balb/c mice (lymphocytes and monocytes). Mice were executed by cervical vertebra luxation and then immersed into 75% ethanol for 1–2 min. The mouse spleen was separated under the sterile condition. It was washed with PBS twice and then the tissues were shredded. They were ground in the cell strainer until only the connective tissue left. The separation liquid with the spleen cells was transferred into the centrifuge tube. The 500 μL RPMI-1640 culture medium was added and 800 g of it was centrifuged for 30 min. The layer of mononuclear cells was sucked out gently. The 1640 culture medium that contained 10% fetal bovine serum was added and it was incubated at 37 °C and 5% CO_2. After 12 h of incubation, the suspended lymphocytes were gently removed to obtain the adherent monocytes. The nonspecific esterase staining (monocytes were positive) was adopted to prove its purity.

THP-1 cells were maintained in the liquid nitrogen. After the cell thawing, The RPMI-1640 culture medium that contained 10% fetal bovine serum was added and it was incubated at 37 °C and 5% CO_2.

2.2.2. ELISA detection and analysis

The cell culture supernatant was collected. The standard well, sample well and blank well were set respectively. The standard substance with the different concentrations was added in the standard 7-well one time. Then the sample serum sample was added. The ELISA plate was covered by the film and it was incubated at 37 °C for 2 h. A total of 100 μL biotinylated primary antibody was added in each well and it was incubated at 37 °C for 1 h. The liquid in the well was removed. Each well was washed with 350 μL cleaning solution. It was immersed for 1–2 min. The plate was washed three times. A total of 100 μL HRP-labeled secondary antibody was added in each well. The ELISA plate was covered by the film and it was incubated at 37 °C for 30 min. A total of 90 μL TMB substrate was added in each well. The ELISA plate was covered by the film. It was placed in a dark place to be colored at 37 °C for 15–25 min. When the first 3–4 of the standard wells appeared to be the obvious gradient blue, the reaction was stopped and then 50 μL 2 M H_2SO_4 was added. Afterwards, the ELISA was used to measure OD value of each well at 492 nm. X axis referred to OD value and Y axis to the log of concentration. The standard curve was drawn and the log of corresponding concentration was obtained from the standard curve according to OD value of the sample. The concentration value was calculated according to the log of concentration. Finally, the actual concentration of the sample would be the product of concentration value and dilution factor.

2.2.3. Real-time PCR

The collected cells were washed with PBS (RNase free). The total RNA extraction kit was used to extract RNA. Qubit Fluorometer system was to detect the concentration and purity of RNA according to the instruction manual of reverse transcription kit. After the reverse transcription of cDNA to total RNA, Real-time PCR was employed to detect the expression of related genes. The mRNA sequence of related gene was queried in NCBI database to design Real-time PCR primer. All primers were synthesized by Shanghai Generay Biotech Co., Ltd., with the specific sequences shown in Table 1. The double ΔCt method was employed to calculate the relative expression of target gene: the mean of three parallel repeated tests was treated as the Ct value of each sample, namely ΔCt = Ct (target gene) Ct (reference), ΔΔCt = ΔCt (sample) − ΔCt (control). Therefore, the relative expression of target gene = $2^{-\Delta\Delta Ct}$ and the relative expression for the control group was $2^0 = 1$. The PCR reaction system was shown in Table 2, where the concentration of primers was decided by the different conditions of gene amplification.