EFFECTS OF PROPOFOL ON THE DIFFERENTIATION OF NORMAL Spleen Dendritic Cells to Immune Cells and Its MECHANISM OF ACTION

XUANYI PAN¹, LILI YUAN², MEILI YANG³, CAIHUA LIU⁴*

¹Department of Anaesthesiology, Affiliated Hospital of Hebei University, Hebei, 071000, China
²Department of Anaesthesiology, Wuhan No. 5 Hospital, Hubei, 430050, China
³Baoding Institute of Food and Drug Control, Hebei, 071000, China
⁴Department of Anaesthesiology, The Central Hospital of Wuhan, 430014, China

*corresponding author: panxuany@sohu.com

Abstract

Based on reports regarding the effect of propofol, in different concentrations on the differentiation of normal spleen dendritic cells to immune cells, this study aimed to clarify the anti-inflammatory and immunomodulatory effect of propofol. Spleen dendritic cells were selected as research material, isolated from male mice. Propofol in different concentrations was cultured with the dendritic cells stimulated with lipopolysaccharide (LPS). The expression of spleen dendritic cells during differentiation in different cell groups was detected and analysed by flow cytometry. Propofol in different concentrations and LPS had low influence on the survival rate of spleen dendritic cells. It inhibited the expression of inflammatory cytokines and significantly lowered the expression level of dendritic cells during differentiation in a dose-dependent manner.

Rezumat

Prezentul studiu îi propune să clarifice efectul antiinflamator și imunomodulator al propofolului având ca punct de pornire rezultatele studiilor care arată că acesta afectează diferențierea celulelor splenice dendritice normale în celule imune, într-o manieră doză-dependență. Studiul a fost efectuat pe celeule splenice dendritice izolate de la șoareci masculi. Propofolul în diferite concentrații a fost adăugat în mediul de cultură al celulelor dendritice tratate cu lipopolizaharide (LPZ). Expresia acestora în timpul diferențierii a fost detectată și analizată prin citometrie în flux. Propofolul în diferite concentrații și LPZ au o influență foarte scăzută asupra ratei de supraviețuire a celulelor splenice dendritice. De asemenea propofolul în anumite doze poate inhiba expresia citokinelor inflamatorii și scade semnificativ gradul de exprimare a celulelor dendritice stimulate de LPZ în timpul diferențierii.

Keywords: propofol, dendritic cells, expression of inflammatory cytokines, lipopolysaccharide, cell differentiation, immunomodulation

Introduction

Propofol, a commonly used intravenous anaesthetic both for the induction and the maintenance of systemic anaesthesia, has also many other non-anesthetic effects such as anti-vomiting, antioxidant, neuroprotective and anti-anxiety [5, 10]. Dendritic cell, a kind of important immune cell, plays a vital role in inflammation reaction. When receiving stimulus, immature dendritic cells are recruited to the inflamed site and may transfer to secondary lymphoid organs such as lymph nodes and induce an immune response. DCs are a highly heterogeneous cell population which contains several subsets with distinct origins, locations, markers, and migratory and functional properties. Growing evidence suggests different DC subsets, either naturally arising or experimentally induced, play critical roles in the maintenance of immune homeostasis via induction of immune tolerance and regulation. The regulatory capacity of DCs depends on their immature state and can be induced by immunosuppressive mediators, genetic manipulation, certain pathogenic stimuli, signals from immune cells or apoptotic cells, and tissue or tumour microenvironment. Regulatory DCs (DCreg) retain the ability of presenting antigens to antigen-specific T cells [13]. Lipopolysaccharides (LPS), important components of bacterial cell walls can stimulate dendritic cells to participate in immunoreaction [18]. Considering the vital position and effects of dendritic cells in immunity, we can infer that the mechanism of propofol in immunomodulation is explained by its effect on dendritic cells [16].

LPS can affect dendritic cells through Toll-like receptor 4 (TLR4). Some studies concerning neuro-immune network have found that, immune cells can express some neurotransmitter receptors such as β1 adrenergic receptors which are correlated with pro-inflammatory response of mononucleosis; β1 adrenergic receptor can effectively restrain the
inflammatory reactions of patients with severe disease conditions [4, 11]. Some experts also made experimental studies on the effect of propofol on normal spleen dendritic cells. In 2009, Vargo J.J. et al. [21] pointed out that propofol could restrain the promotion effect of LPS on the secretion of prostaglandin by dendritic cells. In 2012, Yue L. et al. [23] showed that the dysfunction of dendritic cells was in a direct correlation to poor prognosis of sepsis model. This study mainly evaluated whether propofol in certain concentrations could affect the inflammatory reactions of spleen dendritic cells induced by LPS. From the perspective of LPS-TLR4 pathway, the study preliminarily explored the effect of propofol on dendritic cells and evaluated the anti-inflammatory property of propofol. This work provides theoretical and practical basis for the reasonable use of propofol on patients.

Materials and Methods

Animals. Male mice, aged six weeks and weighed 20 g, were selected for the experimental purposes. One week before the experiment, the mice were put into animal rooms at temperature of 20 ± 3°C and relative humidity of 50 ± 10%. The mice used were purchased from the Experimental Animal Centre of Hebei province. All experimental operations followed the national guidance on the administration and use of experimental animals. Besides, the experiment had been approved by the National Ethics Committee.

Instruments. Instruments used included biosafety cabinet, FACS Calibur flow cytometry (BD Biosciences Immunocytometry Systems, San Diego, CA), refrigerated high-speed centrifuge, biological purification table, optical microscope (Thermo Scientific Inc., USA), low-temperature refrigerator (-20°C), constant temperature water box, electronic balance, centrifuge tube, cell culture plate, photometer, magnetic bead classifier.

Reagents. Reagents used included phosphate buffer solution (PBS) (Thermo Scientific Inc., USA), Dulbecco's modified eagle medium (DMEM), LPS (Sigma Inc., USA), foetal calf serum (Wuhan Procell Life Technology Co., Ltd., China), collagenase D (Roche Group, Switzerland), propofol (Jinan Shengqi Pharmaceutical Co., Ltd., China), mouse percoll (Beijing Tongli Marine Biotechnology Co., Ltd., China), anti-β1 adrenergic receptor antibodies (Shanghai Bangjing Industrial Co., Ltd., China), absolute ethyl alcohol (Wuhan Yuancheng Gongchuang Science and Technology Co., Ltd., China), endotoxin (Lanzhou Institute of Biological Products), Fc receptor inhibitor which is composed of purified Rat anti-mouse CD16/CD32 (Tianjin Sungen Biotech Co., Ltd., China), biotin (Henan Lycang Chemical Co., Ltd., China) labelled mouse dendritic cells aggregation and fluorescein isothiocyanate labelled anti-mouse CD80 and major histocompatibility complex class II molecules (MHC II) (Beijing Baiaolaibo Co., Ltd., China), Toll-like receptor 4 (TLR4) (BioVision Inc., China), Quantikine Mouse IL-10 Immunoassay kit (R&D Systems) (Nanjing Cobiore Co., Ltd., China), and mouse microballoon (Shenzhen Newborn Scientific Apparatus Co., Ltd., China).

Separation and extraction of spleen dendritic cells of mice. Before the experiment, mice were killed and the spleen was removed from each mouse on a sterile super clean bench and put into a sterile plate. PBS and collagenase D were added to cover the spleens. Lymphocyte separation medium was put into a sterile centrifuge tube and then the filtered liquor obtained after the grinding of spleens was added into the centrifuge tube. The filtered liquor should be above the surface of lymphocyte separation medium. Then the centrifuge tube was put into a centrifugal machine. Through centrifugation, the liquid was divided into three layers. Floccule in the middle layer was removed by a straw and then transferred to a new tube. Besides, if the PBS volume was at least 10 times of the floccules was also put into the new tube. Monocyte suspension was obtained after 10-min centrifugation.

Separation of dendritic cells using magnetic bead selection. The collected cell suspension was added with 0.2 µg of CD45RB PE, and the reaction volume was 100 µL. Then it was cultured in a dark place at room temperature for 15 min. After the addition of 1 mL buffer, it was centrifuged for 15 min. The supernatant was removed. 70 µL of buffer and 20 µL of anti-PE micro-balloon were added, followed by mixing and 15 min cultivation. After the addition of 1 mL buffer, it was centrifuged for 15 min. The supernatant was removed. Then 500 µL buffer were added for resuspension. The cell suspension obtained was separated using a magnetic bead classifier to collect outflow cells.

Spleen dendritic cell groups. There were totally eight cell groups, i.e., normal control group, propofol stimulus groups (5 µg/mL group, 10 µg/mL group and 20 µg/mL group), LPS stimulus group (1 µg/mL LPS) and LPS + propofol stimulus groups (1 µg/mL LPS + 5 µg/mL propofol group, 1 µg/mL LPS + 10 µg/mL propofol group and 1 µg/mL LPS + 20 µg/mL propofol group). Before cell plating, LPS was mixed with propofol in the specified concentrations. A sterilized 96-well culture plate was taken and implanted with the spleen dendritic cells. Each well was added with culture solution which contained 10% of foetal calf serum and 90% of DMEM in the corresponding volume till the final volume became 500 µL. Another 96-well culture plate was used for the cell cultivation of the normal control group, the LPS
stimulus group and the 1 µg/mL LPS + propofol stimulus group. After cell plating, the plates were preserved in a sterile carbon dioxide incubator (37°C) overnight. After the removal of supernatant, fresh culture solution containing 10% of foetal calf serum and 90% of DMEM was added for 8-h cell cultivation.

Detection of interleukin (IL)-10 mRNA levels. The cell culture fluid was detected using Quantikine Mouse IL-10 Immunoassay kit (R&D Systems) after centrifugation, followed by the addition of 50 µL of supernatant, 3 hours of membrane covering, 5 min of drying and addition of substrate developer. After being cultured in dark for 30 min, the fluid was added with 100 µL of stop buffer. The absorbance was detected by a microplate reader. The wavelength of IL-10 was calculated according to the standard curve.

Flow cytometry. The spleen dendritic cells were resuspended by PBS using fluorescence staining. Then antibodies including CD45RB-PE, CD11c-APC, CD80-FITC, MHC II-FITC, TLR4 and β1 adrenergic receptor were added. Finally, the fluid was detected by a flow cytometry after centrifugation.

Results and Discussion

Effect of propofol on normal spleen dendritic cells. The results of the flow cytometry experiment demonstrated that, dendritic cells were divided into CD11c<sup>high</sup>CD45RB<sup>low</sup>, CD11c<sup>low</sup>CD45RB<sup>high</sup> and CD11c<sup>low</sup>CD45RB<sup>low</sup>, and they were all regulatory dendritic cells.

The effect of propofol in a concentration of 5 µg/mL and 10 µg/mL on the proportion of CD11c<sup>high</sup>CD45RB<sup>low</sup>, CD11c<sup>low</sup>CD45RB<sup>high</sup> in the propofol stimulus group was not significantly different compared to the control group (p > 0.05).

Propofol in a concentration of 20 µg/mL (the highest dose) significantly lowered the proportion of traditional dendritic cells, but increased the proportion of regulatory dendritic cells, and the differences had statistical significance.

![Figure 1](image1.png)

Proportion of traditional dendritic cells and regulatory dendritic cells

Note: * p < 0.05 compared to the normal control group.

Effects of stimulus of propofol in different concentrations and LPS in a concentration of 1 µg/mL on the survival rate of dendritic cells (Figure 2). It can be seen from Figure 2 that the combination of propofol in three different concentrations and LPS in a concentration of 1 µg/mL had low effect on the growth activity of dendritic cells; though the growth activity was lower compared to the normal control group, the decline was insignificant. It can be seen the growth activity of cells was not affected under the stimulus of propofol in different concentrations and LPS.

![Figure 2](image2.png)

Effects of stimulus of propofol in different concentrations and LPS on the growth activity of dendritic cells
Effect of propofol on the expression level of interleukin (IL)-10 mRNA induced by LPS in a concentration of 1 µg/mL (Figure 3).

It can be seen from Figure 2 that the expression level of IL-10 mRNA had significant increase after being processed with LPS in a concentration of 1 µg/ml, and the difference had statistical significance (p < 0.05); the propofol with different concentrations added had no significant effect on the expression level of IL-10 mRNA (p > 0.05).

Effect of propofol on differentiation of LPS activated dendritic cell. The flow cytometric analysis on the stimulus of LPS in a concentration of 1 µg/mL and 0 µg/mL on dendritic cells suggested that, the differentiation of $CD11c^{high}CD45RB^{low}$ was increased compared with the differentiation of $CD11c^{low}CD45RB^{high}$. Comparisons were made between the 1 µg/mL LPS + 5 µg/mL propofol group, 1 µg/mL LPS + 10 µg/mL propofol group, 1 µg/mL LPS + 20 µg/mL propofol and 1 µg/mL LPS group to study the effect of propofol on the differentiation of dendritic cells activated by LPS. Results demonstrated that, with the increase of the propofol concentration, the proportion of traditional dendritic cells gradually decreased, and the difference had statistical significance (p < 0.05); the decrease in the 1 µg/mL LPS + 20 µg/mL propofol group was the most significant (Table I).

Table I
The proportion of traditional dendritic cells in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Proportion of traditional dendritic cells (mean ± standard deviation)</th>
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<tr>
<td>1 µg/mL LPS</td>
<td>29.9 ± 2.1</td>
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<tr>
<td>1 µg/mL LPS + 5 µg/mL</td>
<td>27.5 ± 2.6*</td>
</tr>
<tr>
<td>1 µg/ml LPS + 10 µg/mL propofol</td>
<td>26.6 ± 2.7*</td>
</tr>
<tr>
<td>1 µg/mL LPS + 20 µg/mL propofol</td>
<td>25.4 ± 3.5*</td>
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* p < 0.05 vs. 1 µg/mL LPS group.

The expression level of TLR4 and β1 adrenergic receptor

It can be seen from Figure 4 that, compared to the normal control group, the LPS stimulus group had a remarkably higher expression level of TLR4 receptor, and the difference had statistical significance (p < 0.05); propofol in different concentrations significantly lowered the expression level of TLR4 which increased...
Propofol may induce immunosuppressive and anti-inflammatory effects on normal spleen dendritic cells. Used in reasonable, well-established concentrations is of high importance for severely ill patients, especially with sepsis. Future uses of propofol in therapy, as well as its action mechanism remain to be further researched.

References


