ORIGINAL ARTICLE

EFFECTS OF PROPOFOL ON THE DIFFERENTIATION OF NORMAL SPLEEN DENDRITIC CELLS TO IMMUNE CELLS AND ITS MECHANISM OF ACTION

XUANYI PAN[1], LILI YUAN[2], MEILI YANG[3], CAIHUA LIU[4]*

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

**corresponding author: panxuany@sohu.com*

Manuscript received: December 2016

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 dentritic 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 își 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-dependentă. Studiul a fost efectuat pe celule 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 concetrații și LPZ au o influență foarte scăzută asupra ratei de supravițuire 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-anaesthetic 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 dendridic 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 Bio-sciences 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 Medical Technology 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 Sungene Biotech Co., Ltd., China), biotin (Hubei Lvcang 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 Cobioer 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 $CD11c^{high}CD45RB^{low}$, $CD11c^{low}CD45RB^{high}$ and $CD11c^{low}CD45RBlow$, 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 $CD11c^{high}CD45RB^{low}$, $CD11c^{low}CD45RB^{high}$ 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.

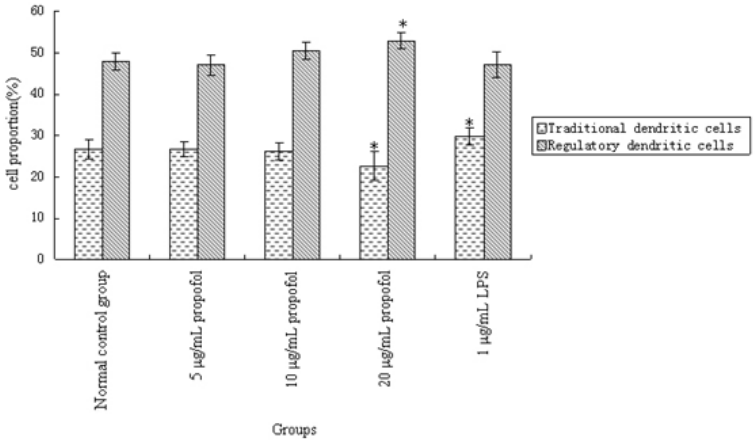

Figure 1.
Proportion of traditional dendritic cells and regulatory dendritic cells
Note: * $p < 0.05$ compared to the normal control group.

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.

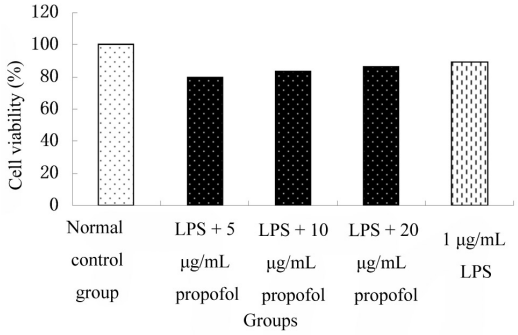

Figure 2.
Effects of stimulus of propofol in different concentrations and LPS on the growth activity of dendritic cells

Effect of propofol on the expression level of interleukin (IL)-10 mRNA induced by LPS in a concentration of 1 μg/mL *(Figure 3).*

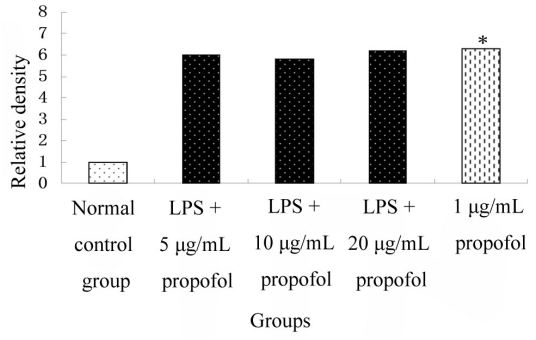

Figure 3.
Effect of propofol in different concentrations on the expression levels of interleukin (IL)-10 mRNA induced by LPS
Note: * $p < 0.05$ compared to the normal control group.

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

Group	Proportion of traditional dendritic cells (mean ± standard deviation)
1 μg/mL LPS	29.9 ± 2.1
1 μg/mL LPS + 5 μg/mL	27.5 ± 2.6*
1 μg/ml LPS + 10 μg/mL propofol	26.6 ± 2.7*
1 μg/mL LPS + 20 μg/mL propofol	25.4 ± 3.5*

* $p < 0.05$ *vs.* 1 μg/mL LPS group.

The expression level of TLR4 and β1 adrenergic receptor

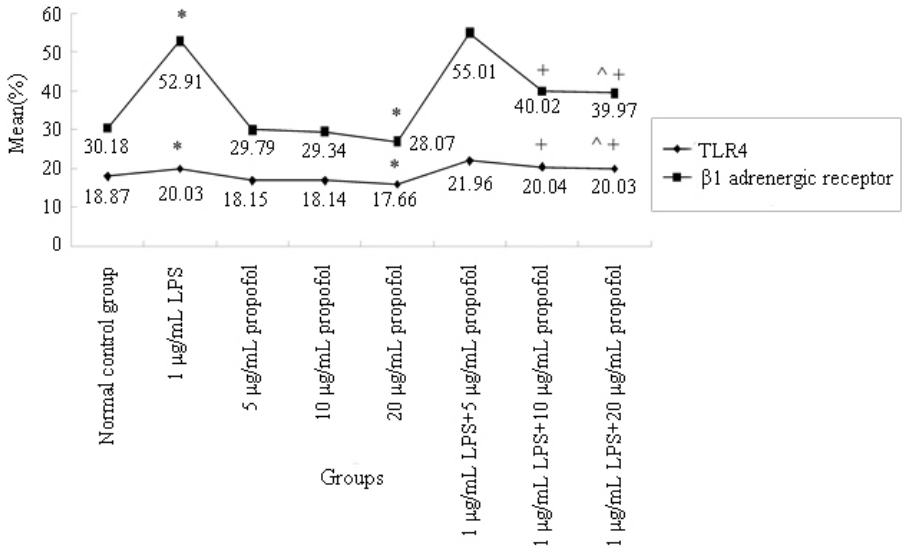

Figure 4.
Different expression levels of TLR4 and β1 adrenergic receptor
* $p < 0.05$ vs. normal control group; + $p < 0.05$ vs. 1 μg/mL LPS group; ^ $p < 0.05$ vs. 1 μg/mL LPS + 5 μg/mL propofol group.

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

previously; but LPS + 5 μg/mL propofol improved the expression level of TLR4. Besides, it was also found after staining the cells with fluorescence labelling and under a fluorescence microscope that, β1 adrenergic receptor actually affected the expression level of spleen dendritic cells; and there was an important difference between the LPS group and normal control group; the effect of propofol in the 1 μg/mL LPS + 5 μg/mL group was the most apparent ($p < 0.05$).

In recent years, the effect of anaesthetics on inflammatory reactions has been of highly concern. Dendritic cells are important inflammatory immune cells and relevant research has become increasingly deeper. The present study proves that propofol can produce important effects on the immune system. Though normal spleen dendritic cells are important components of the congenital immunity, it can develop adaptive immunoreactions by transferring antigen specific signals [17, 22]. It has been reported that the number of dendritic cells in patients who die of sepsis is lesser than that of patients who survive sepsis; therefore the effect of propofol on normal spleen dendritic cells may offer important references for the reasonable use of propofol in patients with sepsis determined by surgical site infections with different germs alone or complicated with fungus strains such as *Fusarium, Candida* and sepsis associated with pleurisy produced by hospital germs [1-3, 7, 8, 19, 20]. For patients with low immunity of different causes, such as administration of immunosuppressive drugs or different pathologies like hepatic viral infections or Human Immunodeficiency Virus (HIV) infections, a special concern should be addressed when using propofol [9]. In this study, male mice were used and spleen dendritic cells were isolated. We established the effect of propofol on the differentiation of normal dendritic cells in a dose depending manner [12, 15]. Besides, it was also found that, LPS in a dose of 1 μg/mL increased the proportion of traditional dendritic cells and moreover activated dendritic cells. Regarding the anti-inflammatory effect of propofol, results demonstrated that it can inhibit specific cell factors involved such as the expression levels of (IL)-10 mRNA, TLR4 and β1 adrenergic receptor [6, 14].

Conclusions

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

1. Alkhamis T., Ivić D., Wagner J., Ivić J., Dobrošević B., Turina I., Kralik K., Barbić J., Postoperative immunosuppression markers and the occurrence of sepsis in patients with benign and malignant disease. *Wien Klin. Wochenschr.*, 2014; 126(23-24): 774-784.
2. Calina D., Rosu L., Rosu A.F., Ianosi G., Ianosi S., Zlatian O., Mitrut R., Docea A.O., Rogoveanu O., Mitrut P., Nicolae A.C., Dragoi C.M., Gofita E., Etiological diagnosis and pharmacotherapeutic management of parapneumonic pleuresy. *Farmacia* 2016; 64(6): 946-952.
3. Călina D., Docea A.O., Rosu L., Zlatian O., Rosu A.F., Anghelina F., Rogoveanu O., Arsene A.L., Nicolae A.C., Drăgoi C.M., Tsiaoussis J., Tsatsakis A.M., Spandidos D.A., Drakoulis N., Gofita E., Antimicrobial resistance development following surgical site infections. *Mol. Med. Rep.*, 2016; 15: 681-688.
4. Chai Z., Gatti S., Toniatti C., Poli V., Bartfai T., Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J. Exp. Med.*, 1996; 183(1): 311-316.
5. Chen C., Xu G.H., Li Y.H., Tang W.X., Wang K., Selective impairment of attention networks during propofol anesthesia after gynecological surgery in middle-aged women. *J. Neurol. Sci.*, 2016; 363: 126-131.
6. Chun J., Tosun A., Kim Y.S., Anti-inflammatory effect of corymbocoumarin from *Seseli gummiferum* subsp. *corymbosum* through suppression of NF-κB signaling pathway and induction of HO-1 expression in LPS-stimulated RAW 264.7 cells. *Int. Immunopharmcol.*, 2016; 31: 207-215.
7. Colcea C., Zlatian O.M., Rosu L., Calina D., Rosu A.F., Chiutu L., Docea A.O., Correlation between consumption of antimicrobials in the intensive care unit and resistance of *Pseudomonas* and *Klebsiella* in pulmonary infection. *Eur. J. Hosp. Pharm.*, 2014; 21(A16): 2047.
8. Cristea O.M., Zlatian O.M., Dinescu S.N., Bălăşoiu A.T., Avramescu C., Bălăşoiu M., Niculescu M., Calina D., A comparative study on antibiotic resistance of *Klebsiella* strains from surgical and intensive care wards. *Curr. Health Sci.*, 2016; 42(2): 169-179.
9. Docea A.O., Gofita E., Calina D., Zaharie S.I., Valcea D.I., Mitrut P., Autoimmune disorders due to double antiviral therapy with peginterferon and ribavirin in patients with hepatitis C virus infection. *Farmacia*, 2016; 64(4): 605-611.
10. Eshghi A., Mohammadpour M., Kaviani N., Tahririan D., Akhlaghi N., Comparative evaluation of bispectral index system after sedation with midazolam and propofol combined with remifentanil *versus* ketamine in uncooperative during dental procedures. *Dent. Res. J.*, 2016; 13(1): 1-6.
11. Fluitman S., Denys D., Vulink N., Schutters S., Heijnen C., Westenberg H., Lipopolysaccharide-induced cytokine production in obsessive-compulsive disorder and generalized social anxiety disorder. *Psychiat. Res.*, 2010; 178(2): 313-316.

12. Gui B., Hua F., Chen J., Xu Z., Sun H., Qianet Y., Protective effects of pretreatment with oleanolic acid in rats in the acute phase of hepatic ischemia-reperfusion injury: role of the PI3K/Akt pathway. *Mediators of Inflammation*, 2014; 2014(1): 143-146.
13. Liu J., Cao X., Regulatory dendritic cells in auto-immunity: A comprehensive review. *J. Autoimmun.*, 2015; 63: 1-12.
14. Ma L., Wu X.Y., Zhang L.H., Chen W.M., Uchiyama A., Mashimo T., Fujino Y., Propofol exerts anti-inflammatory effects in rats with lipopoly-saccharide-induced acute lung injury by inhibition of CD14 and TLR4 expression. *Braz. J. Med. Biol. Res.*, 2013; 46(3): 1-7.
15. Marques C.T., Azevedo T.C.G.D., Nascimento M.S., Medeiros V.P., Alves L.G., Benevides N.M.B., Rocha H.A.O., Leite E.L., Sulfated fucans extracted from algae *Padina gymnospora* have anti-inflammatory effect. *Rev. Bras. Farmacogn.*, 2012; 22(1): 115-122.
16. Merad M., Sathe P., Helft J., Miller J., Mortha A., The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Ann. Rev. Immunol.*, 2013; 31(5): 563-604.
17. Peng M., Ye J.S., Wang Y.L., Chen C., Wang C.Y., Posttreatment with propofol attenuates lipopoly-saccharide-induced up-regulation of inflammatory molecules in primary microglia. *Inflamm. Res.: Offic J. Eur. Histam. Res. Soc.*, 2014; 63(5): 411-418.
18. Santos T.M., Franci D., Schweller M., Ribeiro D.L., Gontijo-Coutinho C.M., Matos-Souza J.R., de Carvalho-Filho M.A., Left Ventricle Tissue Doppler Imaging Predicts Disease Severity in Septic Patients Newly Admitted in an Emergency Unit. *J. Emerg. Med.*, 2015 ; 49(6): 907-915.
19. Spyridaki A., Raftogiannis M., Antonopoulou A., Tsaganos T., Routsi C., Baziaka F., Karagianni V., Mouktaroudi M., Koutoukas P., Pelekanou A., Kotanidou A., Orfanos S.E., van der Meer J.W., Netea M.G., Giamarellos-Bourboulis E.J., Effect of clarithromycin in inflammatory markers of patients with ventilator-associated pneumonia and sepsis caused by Gram-negative bacteria: results from a randomized clinical study. *Antimicrob. Agents Chemother.*, 2012; 56(7): 3819-3825.
20. Tanase A., Colita A., Ianosi G., Neagoe D., Branisteanu D.E., Calina D., Docea A.O, Tsatsakis A., Ianosi S.L., Rare case of disseminated fusariosis in a young patient with graft *vs.* host disease following an allogeneic transplant. *Exp. Ther. Med.*, 2016; 12: 2078-2082.
21. Vargo J.J., Cohen L.B., Rex D.K., Kwo P.Y., Position statement: nonanesthesiologist administration of propofol for GI endoscopy. *Hepatology*, 2009; 50(6): 1683-1689.
22. Wang H., Liu G., Fu W., Xu N., Dai T.J., Effect of Nrf2 on propofol inhibiting lipopolysaccharide-induced acute lung injury in mice. *Chin. Pharmacol. Bull.*, 2013; 29(9): 1286-1290.
23. Yue L., Pawlowski M., Dellal S.S., Xie A., Feng F., Otis T.S., Bruzik K.S., Qian H., Pepperberg D.R., Robust photoregulation of GABA(A) receptors by allosteric modulation with a propofol analogue. *Nat. Comm.*, 2012 ; 3(3): 704-707.