MEDICAL SCIENCE MONITOR

MOLECULAR BIOLOGY

e-ISSN 1643-3750
© Med Sci Monit, 2016; 22: 367-372
DOI: 10.12659/MSM.895739

Received: 2015.08.22
Accepted: 2015.09.10
Published: 2016.02.04

Haloperidol Suppresses NF-kappaB to Inhibit Lipopolysaccharide-Induced Pro-Inflammatory Response in RAW 264 Cells

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1 Shunsuke Yamamoto
ABCDEFG 1 Noriyuki Ohta
BDF 2 Atsuhiro Matsumoto
BF 1 Yu Horiguchi
BF 1 Moe Koide
ACDEG 1 Yuji Fujino

1 Department of Anesthesiology and Intensive Care Medicine, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
2 Department of Anesthesiology, Toyonaka Municipal Hospital, Toyonaka, Osaka, Japan

Corresponding Author: Noriyuki Ohta, e-mail: nohta753@hp-icu.med.osaka-u.ac.jp
Source of support: This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science (T254624340)

Background: Haloperidol, a tranquilizing agent, is administered both to treat symptoms of psychotic disorders and to sedate agitated and delirious patients. Notably, haloperidol has been suggested to inhibit the immune response through unknown mechanisms. We hypothesized that the sedative modulates the immune response via NF-κB.

Material/Methods: Using flow cytometry, we analyzed the effects of haloperidol on expression CD80 and CD86 in RAW 264 cells and in primary macrophages derived from bone marrow. Secretion of interleukin (IL)-1β, IL-6, and IL-12 p40 was measured by enzyme-linked immunosorbent assay. In addition, NF-κB activation was evaluated using a reporter assay based on secretory embryonic alkaline phosphatase. Finally, synthetic antagonists were used to identify the dopamine receptor that mediates the effects of haloperidol.

Results: Haloperidol inhibited NF-κB activation, and thereby suppressed expression of CD80, as well as secretion of IL-1β, IL-6, and IL-12 p40. CD80 and IL-6 levels were similarly attenuated by a D2-like receptor antagonist, but not by a D1-like receptor antagonist.

Conclusions: The data strongly suggest that haloperidol inhibits the immune response by suppressing NF-κB signaling via the dopamine D2 receptor.

MeSH Keywords: Haloperidol • Immune System • Macrophages • NF-kappa B • Receptors, Dopamine D2

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/895739

1882 — 5 26

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Haloperidol belongs to a class of drugs used to alleviate symptoms of psychotic disorders [1], and to safely manage agitated and delirious patients [2]. In addition, these tranquilizers affect immunity, and suppress protection against pathogens and inflammation [3,4]. Indeed, haloperidol and other antipsychotic or tranquilizing agents have been reported to increase the incidence of pneumonia in schizophrenic patients [5–7]. In particular, studies suggest that haloperidol inhibits production of inflammatory molecules such as IL-6 and TNF-α [8,9] through unknown mechanisms.

These observations indicate that haloperidol may suppress activated macrophages [1], which produce various inflammatory and co-stimulatory molecules in response to disease or tissue injury. This inhibitory activity is clinically significant, because excessive production of inflammatory molecules can be harmful [10], and may trigger symptoms of sepsis.

Lipopolysaccharide (LPS), a bacterial antigen that binds toll-like receptor 4, is a potent activator of macrophages, and activates NF-κB [11]. In turn, NF-κB is a master regulator of immunity and inflammation, and controls adhesion molecules, chemokines, growth factors, inflammatory enzymes, and pro-inflammatory cytokines such as COX-2, iNOS, IL-1β, IL-6, and TNF-α [12,13]. In this paper, we examine the effects of haloperidol on NF-κB signaling in macrophages, and on the resulting immune response.

Material and Methods

Animals

Female C57BL/6 mice 4-6 weeks of age were purchased from Japan CLEA (Tokyo, Japan), and fed pathogen-free water and food from the Institute of Experimental Animal Sciences, Faculty of Medicine, Osaka University (Suita, Japan). Experiments were executed with approval from the Institutional Animal Care and Use Committee at Graduate School of Medicine, Osaka University (Suita, Japan). Animals were provided care in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Cell isolation and culture

Macrophages were obtained from murine bone marrow cells according to published methods [14], with minor modifications. Briefly, bone marrow was harvested from the femur and tibia. Red blood cells were removed by hypotonic burst, and bone marrow cells were suspended in RPMI1640 medium supplemented with 10% fetal bovine serum albumin, 2 mM L-glutamine, 100 μg mL^{-1} streptomycin, and 100 U mL^{-1} penicillin. Cells were grown at 37°C, 5% CO_2, and 5×10^5 cells mL^{-1}. Cultures were supplemented on day 0 with 20 ng mL^{-1} recombinant murine macrophage colony stimulating factor. On day 3, half of the medium was replaced with fresh medium supplemented with the colony-stimulating factor. On day 5, adherent proliferating macrophages were harvested, purified, and cultured for 24 h at 5×10^5 cells $well^{-1}$, with or without of 10 μM haloperidol. Finally, cells were stimulated with 100 ng mL^{-1} LPS for another 24 h.

RAW 264 cells, which are macrophages isolated from Balb/c mice and immortalized with Abelson leukemia virus, were purchased from RIKEN BioResource Center (Tsukuba, Japan). Cells were cultured in complete media at 37°C, 5% CO_2 and 5×10^4 cells $well^{-1}$. On day 2, cells were treated with 0.5, 5, or 10 μM haloperidol for 24 h, and then exposed to 300 ng mL^{-1} LPS for another 24 h.

Reagents and antibodies

Monoclonal antibodies conjugated to fluorescein isothiocyanate, allophycocyanin, and phycoerythrin were procured from BD Biosciences (San Diego, CA) to measure expression of CD11b (M1/70), CD80 (16-10A1), and CD86 (GL1), respectively. *Escherichia coli* 055: B5 LPS was purchased from Sigma-Aldrich (St Louis, MO), along with haloperidol and SCH23390 and L750.667, which are antagonists of the dopamine D1-like and D2-like receptor, respectively. Haloperidol and L750.667 were dissolved in dimethyl sulfoxide, while SCH23390 was dissolved in distilled water, and the drugs were diluted with culture medium immediately before use.

Flow cytometry

Expression of surface molecules in RAW 264 cells and in primary bone marrow macrophages was analyzed by flow cytometry. Cells were stained with fluorescently labeled antibodies against CD11b, CD80, and CD86, and sorted on a FACSVerse flow cytometer (BD Biosciences, San Diego, CA). Data were analyzed using FlowJo (FlowJo LLC., Ashland, OR).

Enzyme-linked immunosorbent assay

RAW 264 cells were cultured with or without haloperidol, and treated with 100 ng mL^{-1} LPS for 12 h. Commercially available ELISA kits (R&D Systems, Minneapolis, MN) were used following the manufacturer's instructions to measure the concentration of IL-1β, IL-6, and IL-12 p40 in the culture supernatant.

NF-κB activation

The effects of haloperidol on NF-κB activation was investigated using RAW-Blue cells (InvivoGen, San Diego, CA), which

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

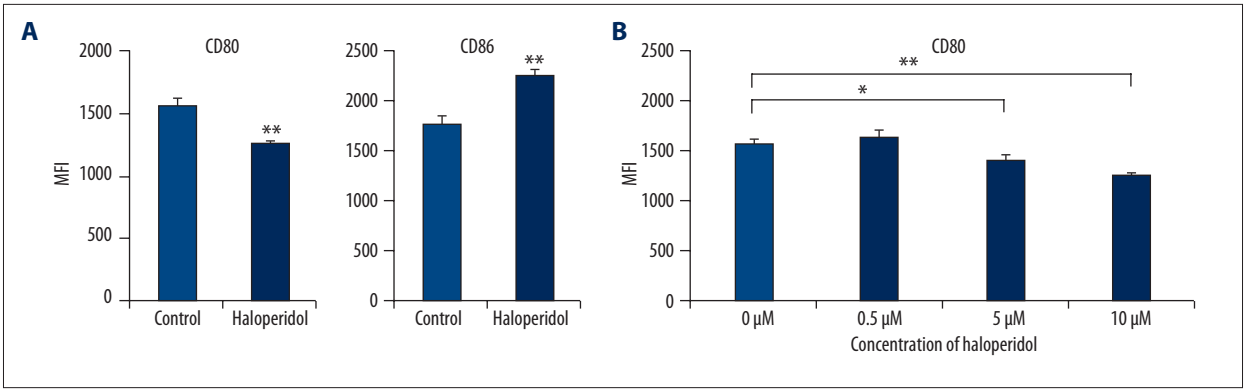

Figure 1. (**A**) Effect of haloperidol on expression of the co-stimulatory molecules CD80 and CD86 in LPS-stimulated RAW 264 cells. Cells were treated with or without haloperidol on day 2 of culture, stimulated with 300 ng ml^{-1} LPS on day 3, and analyzed by flow cytometry on day 4. CD80 and CD86 expression was measured in CD11b-gated cells. Data are mean ±SD (n=6). ** P<0.01. (**B**) Haloperidol suppresses CD80 expression in a dose-dependent fashion. * P<0.05; ** P<0.01. MFI, mean fluorescence intensity.

contain a reporter vector expressing secretory embryonic alkaline phosphatase under the control of an NF-κB/AP1-inducible promoter. Cells were cultured at 1×10^5 cells well^{-1} in complete medium containing 200 µg mL^{-1} zeocin, and stimulated with 300 ng mL^{-1} LPS for 24 h, with or without haloperidol. Cells were stained with QUANTI-Blue (InvivoGen, San Diego, CA), and production of the alkaline phosphatase was measured by absorbance at 655 nm.

Activity of D1-like and D2-like receptor antagonists

RAW 264 cells were cultured at 5×10^4 cells well^{-1} in 24-well plates. On day 2, cells were treated with 10 µM SCH23390 or 10 µM L750.667 for 24 h, and then treated with 300 ng mL^{-1} LPS for another 24 h. We analyzed expression of CD80 and secretion IL-6 by flow cytometry and ELISA, respectively.

Statistical analysis

Statistical analyses were conducted in JMP Pro Version 11.2 (SAS Institute, Cary, NC) and in Power and Sample Size Calculations Version 3.1.2 (Vanderbilt School of Medicine, Nashville, TN). Data are reported as mean ±SD. Means were compared by two-tailed Student's t test, ANOVA, or Bonferroni test. P<0.05 was considered statistically significant.

Preliminary experiments were executed to estimate effect size, and determine sample size. In these experiments, we measured a continuous response variable with one or more controls per treatment, and found that the response was normally distributed within each group. Based on the observed standard deviation and difference in means, four experimental groups and four control groups would be required to reject the null hypothesis that the means are equal with a probability (power) of 0.8. The Type I error probability associated with this test of the null hypothesis is 0.01. Thus, six samples per group were used in future experiments.

Results

Haloperidol suppresses LPS-induced upregulation of macrophage surface markers

Preliminary experiments indicated that haloperidol does not reduce viability in RAW 264 cells up to 10 µM (data not shown). Moreover, preliminary experiments confirmed that LPS induced expression of the co-stimulatory molecules CD80 and CD86 (data not shown). Treatment with 10 µM haloperidol blocked the LPS-induced increase in CD80 in a dose-dependent manner (Figure 1A, 1B). However, the drug did not prevent the upregulation of surface CD86 (Figure 1A). In primary cultures of macrophages derived from bone marrow, haloperidol also reversed the effects of LPS on both CD80 and CD86 (Figure 2).

Haloperidol inhibits secretion of pro-inflammatory cytokines from RAW 264 cells

Macrophages secrete pro-inflammatory cytokines such as IL-1β, IL-6, and IL-12p40 in response to LPS. Thus, we measured secretion of these cytokines from RAW 264 cultures exposed to both LPS and haloperidol. Notably, the sedative inhibited LPS-induced secretion (Figure 3).

Haloperidol blocks NF-κB activation

NF-κB controls the transcription of pro-inflammatory cytokines and macrophage surface molecules. Therefore, a reporter assay based on secretory embryonic alkaline phosphatase was used to test whether the ability of haloperidol to suppress

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

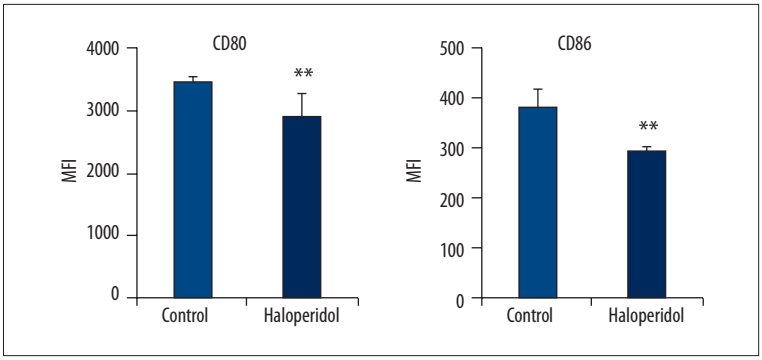

Figure 2. Haloperidol suppresses LPS-induced expression of CD80 and CD86 in primary macrophages derived from bone marrow. Cells were treated with or without haloperidol on day 5, and with 100 ng mL^{-1} LPS on day 6. Expression of CD80 and CD86 was measured by flow cytometry in CD11b-gated cells 24 h after LPS treatment. Data are mean ±SD (n=6). ** *P*<0.01. MFI, mean fluorescence intensity.

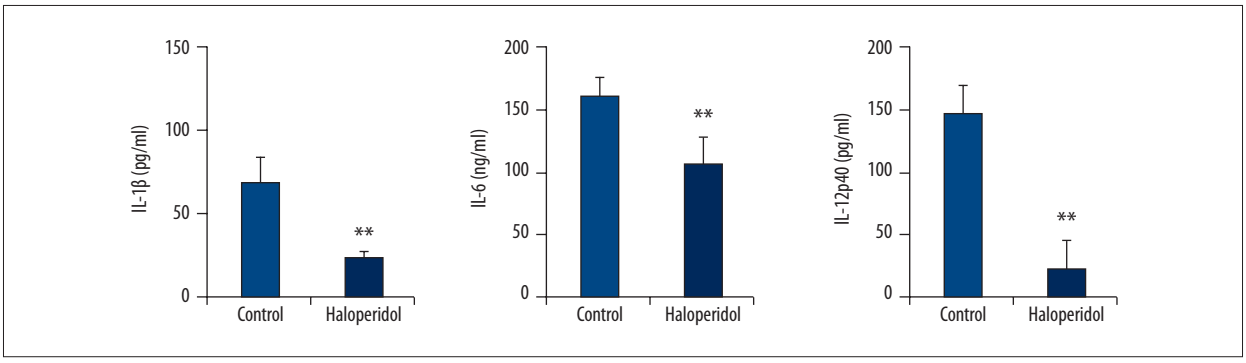

Figure 3. Secretion of IL-1β, IL-6, and IL-12 p40 in RAW 264 cells treated with or without haloperidol on day 2 and exposed to 300 ng mL^{-1} LPS on day 3. The culture supernatant was collected 24 h after LPS induction, and levels of IL-1β, IL-6, and IL-12 p40 were measured by enzyme-linked immunosorbent assay. Data are mean ±SD (n=6). ** *P*<0.01.

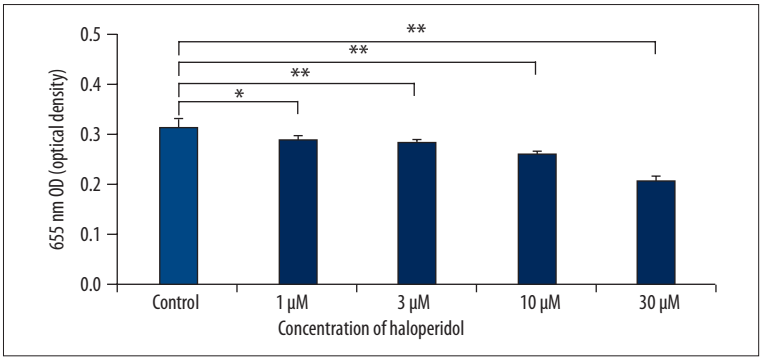

Figure 4. NF-κB activation in RAW-Blue cells cultured with or without haloperidol at 1×10^5 cells well^{-1}, and stimulated for 24 h with 300 ng ml^{-1} LPS. Expression of the reporter enzyme secretory embryonic alkaline phosphatase was measured by colorimetry at 655 nm. Data are mean ±SD (n=6). * *P*<0.05; ** *P*<0.01.

inflammation depends on NF-κB activity. Alkaline phosphatase activity increased in RAW-Blue cells after exposure to LPS, confirming that the antigen elicits NF-κB signaling. Notably, treatment with haloperidol suppressed NF-κB activation in a concentration-dependent manner (Figure 4).

An antagonist of dopamine D2-like receptor prevents activation of RAW 264 cells

We hypothesized that a dopamine receptor mediates the ability of haloperidol to suppress immune response. To test this hypothesis, RAW 264 cells were cultured for 24 h with SCH23390 or L750.667, which are antagonists of the dopamine D1-like and D2-like receptor, respectively. LPS-induced expression of CD80 was lower in RAW 264 cells treated with L750.667 (Figure 5A). In contrast, SCH23390 did not block the effects of LPS. Similarly, L750.667, but not SCH23390, suppressed IL-6 secretion to the same extent as haloperidol (Figure 5B).

Discussion

Macrophages are central to the immune response. In particular, these cells modulate Th1/Th2 polarization in acquired immunity by expressing co-stimulatory molecules and secreting IL-12, a key trigger of Th1 [15]. Additionally, macrophages secrete inflammatory molecules such as IL-6 and TNF-α to protect against pathogens, especially intracellular pathogens such as Mycobacteria [12].

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

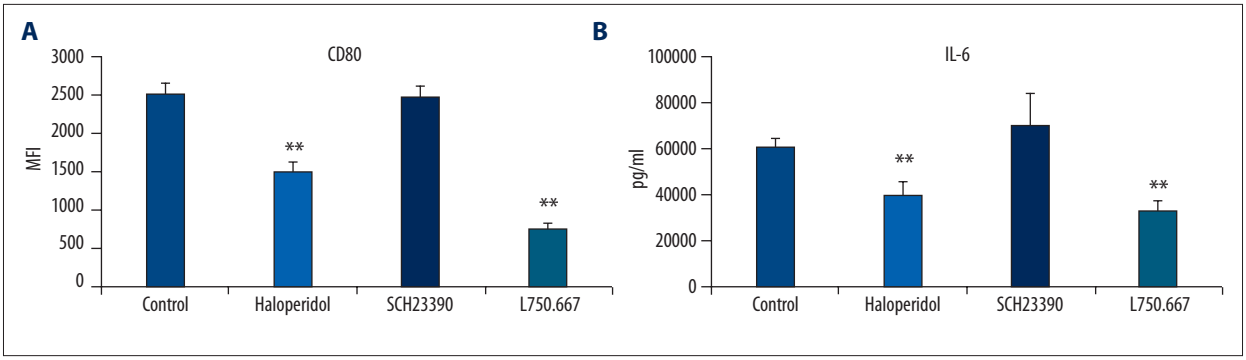

Figure 5. Effect of dopamine D1-like and D2-like receptor antagonists on (**A**) CD80 expression and (**B**) IL-6 secretion in RAW 264 cells exposed to LPS. Cells were treated on day 2 with haloperidol; SCH23390, a D1-like receptor antagonist; and L750.667, a D2-like receptor antagonist. On day 3, RAW 267 cells were stimulated with 300 ng mL^{-1} LPS for another 24 h. Flow cytometry was used to measure CD80 expression in CD11b-gated cells, while enzyme-linked immunosorbent assay was used to measure IL-6 secreted into the culture supernatant. Cells that were treated with LPS, but not with drugs, were used as control. Data are mean ±SD (n=6). ** $P<0.01$.

In particular, macrophages respond robustly to LPS, a main component of the outer membrane in gram-negative bacteria. The antigen triggers expression of cytokines and co-stimulatory molecules [16] through NF-κB [17,18], a master regulator of the inflammatory response [12]. In this study, we report that the sedative haloperidol suppresses LPS-induced expression of co-stimulatory molecules, as well as secretion of inflammatory cytokines from both RAW 264 cells and primary macrophages. This activity appears to depend on the NF-κB pathway, and seems to be regulated by the dopamine D2-like receptor.

There are five subtypes of dopamine receptors, namely D1 to D5. D1 and D5 are grouped into D1-like receptors, while the rest are considered D2-like receptors [19]. In the central nervous system, haloperidol mainly targets D2, but may also bind D1, D4, and D5 [19,20]. Notably, immune cells such as lymphocytes and dendritic cells have been shown to express functional dopamine receptors [21]. Accordingly, our data indicate that an antagonist of D2-like receptors also suppresses inflammation and NF-κB signaling to the same extent as haloperidol, strongly suggesting that the sedative inhibits macrophage activation through D2-like receptors. To the best of our knowledge, this is the first demonstration that haloperidol inhibits inflammation via the D2-like receptor.

The ability of haloperidol to suppress immunity has important clinical implications. For instance, administration of antipsychotics has been reported to exacerbate infections [22], and to increases the incidence of pneumonia among patients with schizophrenia and bipolar disorder [5–7]. Our results provide, for the first time, a potential mechanistic basis for these reports. The ability of the drug to inhibit expression of IL-12 indicates that it switches the immune response away from Th1, which is key to pathogen clearance. As a result, the tranquilizing agent may lower the ability to fight infections.

On the other hand, haloperidol may strongly benefit critically ill patients with delirium, who are at increased risk of morbidity and mortality [23]. The relationship between inflammation and delirium has been previously investigated [24–26]. For example, elevated serum IL-1β and IL-6 have been demonstrated to be associated with delirium [24]. Indeed, it is supposed that pro-inflammatory molecules may injure synapses and induce delirium by facilitating the migration of macrophages into the central nervous system [25,26]. Thus, the ability of haloperidol to suppress secretion of such molecules may prevent or mitigate further adverse events.

This study is limited, first by the use of RAW 264 cells, which are immortalized macrophages that may have different properties as macrophages *in vivo*. Nevertheless, RAW 264 cells are an established model system for macrophage biology. In any case, we observed that haloperidol had the same effect on both RAW 264 cells and primary macrophages from bone marrow. A second limitation is the use of a reporter assay to detect NF-κB activation instead of direct quantification by Western blotting.

Conclusions

In conclusion, we demonstrate that haloperidol can suppress the immune response, possibly by inhibiting NF-κB activation in macrophages via the dopamine D2-like receptor. Further investigations of *in vivo* subsets of murine macrophage are required to clarify the effect of haloperidol on macrophage function.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

References:

1. Singer AJ, Clark RA: Cutaneous wound healing. N Engl J Med, 1999; 341: 738–46
2. Riker RR, Shehabi Y, Bokesch PM et al: Dexmedetomidine *vs.* midazolam for sedation of critically ill patients: a randomized trial. JAMA, 2009; 301: 489–99
3. Sanders RD, Godlee A, Fujimori T et al: Benzodiazepine augmented γ-amino-butyric acid signaling increases mortality from pneumonia in mice. Crit Care Med, 2013; 41: 1627–36
4. Yang SY, Liao YT, Liu HC et al: Antipsychotic drugs, mood stabilizers, and risk of pneumonia in bipolar disorder: a nationwide case-control study. J Clin Psychiatry, 2013; 74: e79–86
5. Trifirò G: Antipsychotic drug use and community-acquired pneumonia. Curr Infect Dis Rep, 2011; 13: 262–68
6. Trifirò G, Gambassi G, Sen EF et al: Association of community-acquired pneumonia with antipsychotic drug use in elderly patients: a nested case-control study. Ann Intern Med, 2010; 152: 418–25, w139–40
7. Song C, Lin Ah, Kenis G et al: Immunosuppressive effects of clozapine and haloperidol: enhanced production of the interleukin-1 receptor antagonist. Schizophr Res, 2000; 42: 157–64
8. Moots RJ, Al-Saffar Z, Hutchinson D et al: Old drug, new tricks: haloperidol inhibits secretion of proinflammatory cytokines. Ann Rheum Dis, 1999; 58: 585–87
9. Duffield JS: The inflammatory macrophage: a story of Jekyll and Hyde. Clin Sci (Lond.), 2003; 104: 27–38
10. van den Boogaard M, Schoonhoven L, van Achterberg T et al: Haloperidol prophylaxis in critically ill patients with a high risk for delirium. Crit Care, 2013; 17: R9
11. Zhang YL, Dong C: MAP kinases in immune responses. Cell Mol Immunol, 2005; 2: 20–27
12. Lawrence T, Willoughby DA, Gilroy DW: Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol, 2002; 2: 787–95
13. Vallabhapurapu S, Karin M: Regulation and function of NF-kappaB transcription factors in the immune system. Annu Rev Immunol, 2009; 27: 693–733
14. Zhang X, Goncalves R, Mosser DM: The isolation and characterization of murine macrophages. Curr Protoc Immunol, 2008; 83: 14.1.1–14
15. Ueno H, Klechevsky E, Morita R et al: Dendritic cell subsets in health and disease. Immunol Rev, 2007; 219: 118–42
16. Matsumoto A, Ohta N, Goto Y et al: Haloperidol suppresses murine dendritic cell maturation and priming of the T helper 1-type immune response. Anesth Analg, 2015; 120: 895–902
17. Obiora E, Hubbard R, Sanders RD, Myles PR: The impact of benzodiazepines on occurrence of pneumonia and mortality from pneumonia: a nested case-control and survival analysis in a population-based cohort. Thorax, 2013; 68: 163–70
18. Ely EW, Shintani A, Truman B et al: Delirium as a predictor of mortality in mechanically ventilated patients in the intensive care unit. JAMA, 2004; 291: 1753–62
19. Ritter C, Tomasi CD, Dal-Pizzol F et al: Inflammation biomarkers and delirium in critically ill patients. Crit Care, 2014; 18: R106
20. Eckenhoff RG, Laudansky KF: Anesthesia, surgery, illness and Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry, 2013; 47: 162–66
21. Terrando N, Eriksson LI, Ryu JK et al: Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol, 2011; 70: 986–95
22. He H, Li W, Chen SY et al: Suppression of activation and induction of apoptosis in RAW264.7 cells by amniotic membrane extract. Invest Ophthalmol Vis Sci, 2008; 49: 4468–75
23. Sanghera JS, Weinstein SL, Aluwalia M et al: Activation of multiple proline-directed kinases by bacterial lipopolysaccharide in murine macrophages. J Immunol, 1996; 156: 4457–65
24. Doyle SL, O'Neill LA: Toll-like receptors: from the discovery of NFkappaB to new insights into transcriptional regulations in innate immunity. Biochem Pharmacol, 2006; 72: 1102–13
25. Missale C, Nash SR, Robinson SW et al: Dopamine receptors: from structure to function. Physiol Rev, 1998; 78: 189–225
26. Sibley DR, Monsma FJ Jr., Shen Y: Molecular neurobiology of dopaminergic receptors. Int Rev Neurobiol, 1993; 35: 391–415

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]