Biochemical and Biophysical Research Communications 458 (2015) 92–97

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Activation of murine microglial N9 cells is attenuated through cannabinoid receptor CB2 signaling

Lei Ma [a, 1], Ji Jia [a, 1], Xiangyu Liu [b, 1], Fuhai Bai [a], Qiang Wang [a, *], Lize Xiong [a, *]

[a] Department of Anesthesiology, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China
[b] Department of Plastic Surgery, Xijing Hospital, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China

ARTICLE INFO

Article history:
Received 5 January 2015
Available online 27 January 2015

Keywords:
Cannabinoid CB2 receptor
Pretreatment
Inflammation
Microglia
Protein kinase C

ABSTRACT

Inhibition of microglial activation is effective in treating various neurological disorders. Activation of microglial cannabinoid CB2 receptor induces anti-inflammatory effects, and the mechanism, however, is still elusive. Microglia could be activated into the classic activated state (M1 state) or the alternative activated state (M2 state), the former is cytotoxic, and the latter is neurotrophic. In this study, we used lipopolysaccharide (LPS) plus interferon-γ (IFNγ) to activate N9 microglia and hypothesized the pretreatment with cannabinoid CB2 receptor agonist AM1241 attenuates microglial activation by shifting microglial M1 to M2 state. We found that pretreatment with 5 μM AM1241 at 1 h before microglia were exposed to LPS plus IFNγ decreased the expression of inducible nitric oxide synthase (iNOS) and the release of pro-inflammatory factors, increased the expression of arginase 1 (Arg-1) and the release of anti-inflammatory and neurotrophic factors in microglia. However, these effects induced by AM1241 pretreatment were significantly reversed in the presence of 10 μM cannabinoid CB2 receptor antagonist AM630 or 10 μM protein kinase C (PKC) inhibitor chelerythrine. These findings indicated that AM1241 pretreatment attenuates microglial activation by shifting M1 to M2 activated state via CB2 receptor, and the AM1241-induced anti-inflammatory effects may be mediated by PKC.

1. Introduction

Microglia, the specialized macrophages, are the major cellular source and target of inflammatory mediators in the central nervous system (CNS) [1,2]. They can be activated during both neuroinflammatory and neurodegenerative disorders, including brain ischemia, trauma, and Alzheimer's disease [3,4]. Activated microglial cells secrete a variety of inflammatory factors, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-10, which contributes to the pathogenesis of neural damage in many CNS diseases [5]. Therefore, inhibiting microglial over-activation may be an important therapeutic approach for these neurological diseases.

However, many studies have demonstrated that two microglia/macrophage states exist (M1 and M2), reflecting the cell polarization [6]. The former is cytotoxic by secreting pro-inflammatory factors, such as TNF-α, IL-1β and IL-6. As a comparison, the latter is beneficial due to inhibiting inflammatory responses by producing anti-inflammatory and neurotrophic factors, including IL-10, brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) [7]. Based on a series of studies about the field of macrophage M1 and M2 states and the fact that microglial cells are the member of macrophage lineage, we chose the markers of macrophage M1 and M2 states to explore microglial polarization. Then, it is a key issue that how to shift M1 to M2 state and alleviate microglial over-activation during neuroinflammation.

Cannabinoid CB2 receptor is a G protein-coupled receptor expressed mainly in microglia and peripheral immune cells, modulating antigen presentation, cytokine/chemokine production, and cell migration [8]. The expression of CB2 receptor was increased in brain injury and many types of neurodegenerative diseases including Alzheimer's disease, amyotrophic lateral

Abbreviations: LPS, lipopolysaccharide; IFNγ, interferon-γ; iNOS, inducible nitric oxide synthase; Arg-1, arginase 1; PKC, protein kinase C; CNS, central nervous system; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor.

* Corresponding authors. Fax: +86 29 8477 1262.
E-mail addresses: wangqiang@fmmu.edu.cn (Q. Wang), mzkxlz@126.com (L. Xiong).

[1] Lei Ma, Ji Jia and Xiangyu Liu contributed equally to this work.

http://dx.doi.org/10.1016/j.bbrc.2015.01.073
0006-291X/

sclerosis, Huntington's chorea [9,10]. Especially, many studies have confirmed that CB2 receptor activation, by using CB2 receptor agonists and/or knockout mice, is protective against cerebral ischemia/reperfusion (I/R) injury by attenuating the endothelial cell activation and inflammatory response, suggesting CB2 receptor may be a target for the treatment of neuroinflammation and neurodegeneration [11,12]. However, whether CB2 receptor activation-induced reduction of inflammatory response is by inhibiting microglial over-activation and regulating M1/M2 states is still unknown. In addition, Xu et al. reported that inhalable anesthetic isoflurane-induced anti-inflammation was mediated by protein kinase C (PKC) in microglial cells exposed to lipopolysaccharide (LPS) plus interferon-γ (IFNγ) [13], and we have ever found that electroacupuncture (EA)-induced neuroprotection against ischemia was also mediated by PKC via endocannabinoid system [14]. However, whether PKC is involved in AM1241 pretreatment-induced anti-inflammatory effects in microglia has not been investigated.

In the present study, we used mouse N9 microglia exposed to LPS plus IFNγ to induce microglial activation and hypothesized that CB2 receptor agonist AM1241 can attenuate the activation of microglia exposed to LPS plus IFNγ by shifting M1 to M2 activated state, and the AM1241-induced anti-inflammation may be mediated by PKC.

2. Materials and methods

2.1. Materials

N9 cells, a mouse microglial cell line (a gift provided by Prof. Yingying Le, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China), were generated by immortalization of E13 mouse embryonic cultures with the 3RV retrovirus. The N9 cells are similar to primary microglial cultures in producing pro-inflammatory substance, including nitric oxide (NO) and various cytokines after stimulation [15]. (R,S)-3-(2-Iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1H-indole (AM1241) and 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl) methanone (AM630) were purchased from Alexis Biochemicals (CH). Iscove's modified Dulbecco's medium (IMDM), fetal bovine serum (FBS), LPS (*Escherichia coli* 055:B5), and recombinant rat IFNγ produced from *E. coli* were purchased from Invitrogen Corporation (Carlsbad, CA, USA). Chelerythrine was obtained from Biomol (Plymouth Meeting, PA, USA).

2.2. Cell culture

The mouse N9 microglial cells were cultured in IMDM medium containing 5% FBS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM glutamine in humidified atmosphere of 95% air and 5% CO_2 at 37 °C. The medium was changed every 3 days. Stock cells were passaged 2–3 times/week with 1:4 split ratio and used within 8 passages.

2.3. Immunohistochemistry

N9 cells were seeded into confocal microscopy special dishes at a density of 2×10^4 cells/dish. After treatment, the cells were then fixed with 4% paraformaldehyde solution for 1 h. The fixed cells were blocked with 50 mg BSA/ml in PBS for 30 min. The cells were then washed three times with PBS, followed by incubation at 4 °C overnight with anti-iNOS (1:1000) or anti-Arg-1 (1:1000) primary antibodies, respectively. At the end of the incubation, 200 μl of DAPI staining solution was added into each dish. Then, the cells were incubated in Cy3-labeled secondary antibody solution (1:800, Invitrogen) or FITC-labeled secondary antibody solution (1:500, Sigma, USA) for 1 h at room temperature. Then, the dishes were washed three times before observation with microscope. Images were captured using a laser confocal microscope (Olympus, Japan).

2.4. Western blot analysis

After different treatments, the total protein was collected. The protein concentration was determined by the Bradford method, and Western blot analysis was performed as previously described [16]. The following primary antibodies were used: anti-iNOS and anti-Arg-1 (1:1000, Chemicon, USA), and anti-GAPDH antibody (1:1000, CWBIO, China). The secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (1:10,000, CWBIO, China) was used. Antigens were detected using the chemiluminescence technique (Amersham Pharmacia Biotech Piscataway, USA). Image analysis was accomplished with the assistance of computerized analysis software (Bio-Rad Laboratories, Hercules, USA).

2.5. Enzyme-linked immunosorbent assay

The supernatants of the cell culture were harvested and measured for cytokine concentrations of IL-1β, IL-6, TNF-α, IL-10, BDNF and GDNF (PeproTech Inc., USA) by using the corresponding quantification enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. Determinations were performed in duplicate and repeated in three independent experiments. The results are expressed in nanograms per liter.

2.6. Statistical analysis

SPSS 13.0 for Windows (SPSS Inc., Chicago, USA) was used to conduct statistical analysis. All values were presented as the means ± SD, and were analyzed by one-way ANOVA. Between-group

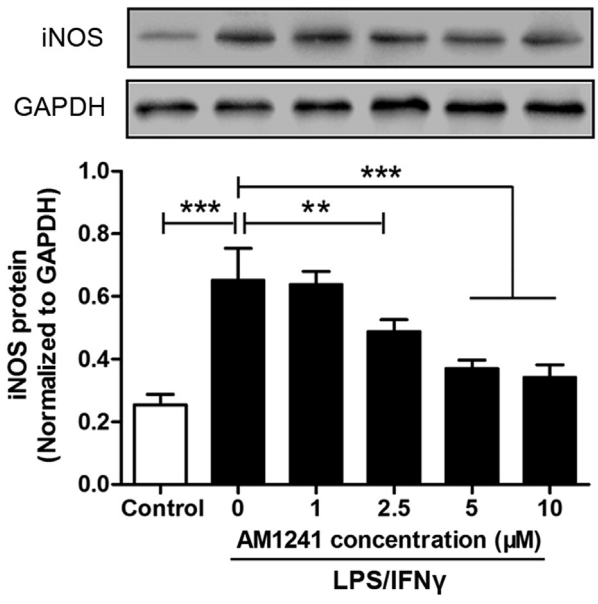

Fig. 1. AM1241 alleviated iNOS expression in N9 microglia exposed to LPS plus IFNγ in a concentration dependent manner. The mouse N9 microglial cells were pretreated with or without various concentrations of AM1241. They were then exposed to 10 ng/ml LPS plus 10 U/ml IFNγ for 24 h at 1 h after AM1241 pretreatment. The expression of iNOS was evaluated by Western blot analysis (n = 6). Data are means ± SD. **: $P < 0.01$; ***: $P < 0.001$.

differences were detected with post-hoc Student–Newman–Keuls tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. AM1241 alleviated microglial activation induced by LPS plus IFNγ

We used selective CB2 receptor agonist AM1241 to investigate whether the activation of CB2 receptor could reduce microglial over-activation. The expression of iNOS was measured as the marker of microglial activation by Western blot analysis. To find a suitable AM1241 concentration, microglia were exposed to AM1241 at 1 μM, 2.5 μM, 5 μM and 10 μM, respectively, at 1 h before the exposure of 10 ng/ml LPS plus 10 U/ml IFNγ for 24 h. The expression of iNOS was significantly decreased in the presence of AM1241 at 2.5 μM ($P < 0.01$), 5 μM ($P < 0.001$) and 10 μM ($P < 0.001$), and 5 μM AM1241 was used in the subsequent experiments (Fig. 1).

3.2. CB2 receptor antagonist reversed the AM1241-induced effects on iNOS and Arg-1 expressions

The M1/M2 states of microglia were assessed by detecting the expressions of M1 marker iNOS and M2 marker Arg-1 at 24 h after the exposure of LPS plus IFNγ. The expressions of iNOS and Arg-1 were analyzed by immunocytochemistry and Western blot analysis. Pretreatment with 5 μM AM1241 for 1 h significantly attenuated iNOS expression (Fig. 2A and C, $P < 0.05$) and increased Arg-1 expression (Fig. 2B and C, $P < 0.05$) in the microglia exposed to LPS plus IFNγ. Co-administration of 10 μM CB2 receptor antagonist AM630 significantly reversed the benefits induced by AM1241 pretreatment ($P < 0.05$), suggesting CB2 receptor may be involved in the AM1241-induced modulation of microglial M1/M2 states in N9 microglia.

3.3. AM1241 decreased pro-inflammatory factor release and increased anti-inflammatory and neurotrophic factor release

Pro-inflammatory factors such as TNF-α, IL-1β and IL-6 are biomarkers of microglial M1 activated state; in contrast, anti-inflammatory factor IL-10 and neurotrophic factors BDNF and GDNF are the biomarkers of M2 state. The levels of TNF-α, IL-1β and IL-6 showed significant increase in the LPS plus IFNγ group compared with the control group ($P < 0.05$). Pretreatment with 5 μM AM1241 attenuated the levels of TNF-α, IL-1β and IL-6 ($P < 0.05$) in the supernatants. The benefits induced by AM1241 were partially reversed in the presence of selective CB2 receptor antagonist, AM630 (Fig. 3A–C, $P < 0.05$). Moreover, AM1241 increased the levels of IL-10, BDNF and GDNF ($P < 0.05$) and the AM1241-induced benefits were also significantly reversed by AM630 (Fig. 3D–F, $P < 0.05$). These findings indicated that AM1241 pretreatment could improve the pro-/anti-inflammatory factors balance between microglial M1 and M2 activated state.

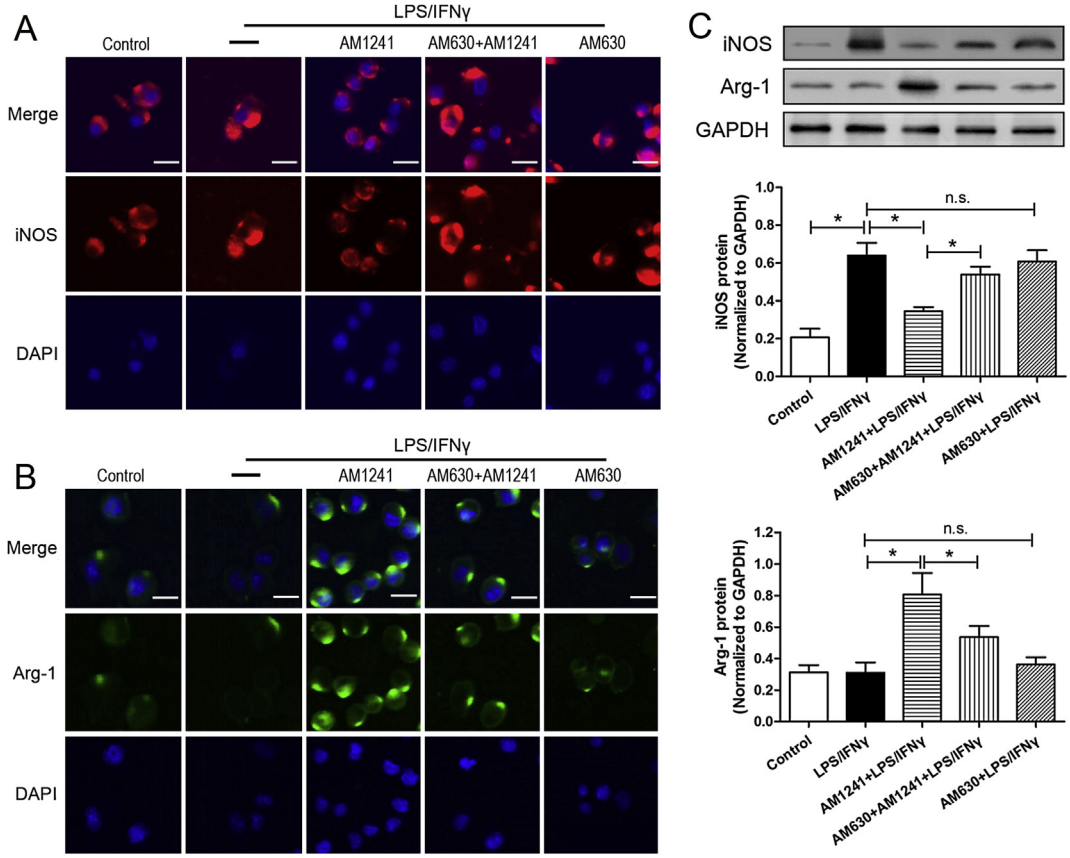

Fig. 2. AM1241 pretreatment decreased iNOS expression and increased Arg-1 expression via CB2 receptor in N9 microglia. The mouse N9 microglial cells were pretreated with or without 5 μM AM1241 in the presence or absence of 10 μM CB2 antagonist AM630. They were then exposed to 10 ng/ml LPS plus 10 U/ml IFNγ for 24 h at 1 h after AM1241 pretreatment. The expressions of iNOS (A and C) and Arg-1 (B and C) were analyzed by immunocytochemistry and Western blot analysis (n = 6). Scale bars = 10 μm. Data are means ± SD. *: $P < 0.05$; n.s.: no significance.

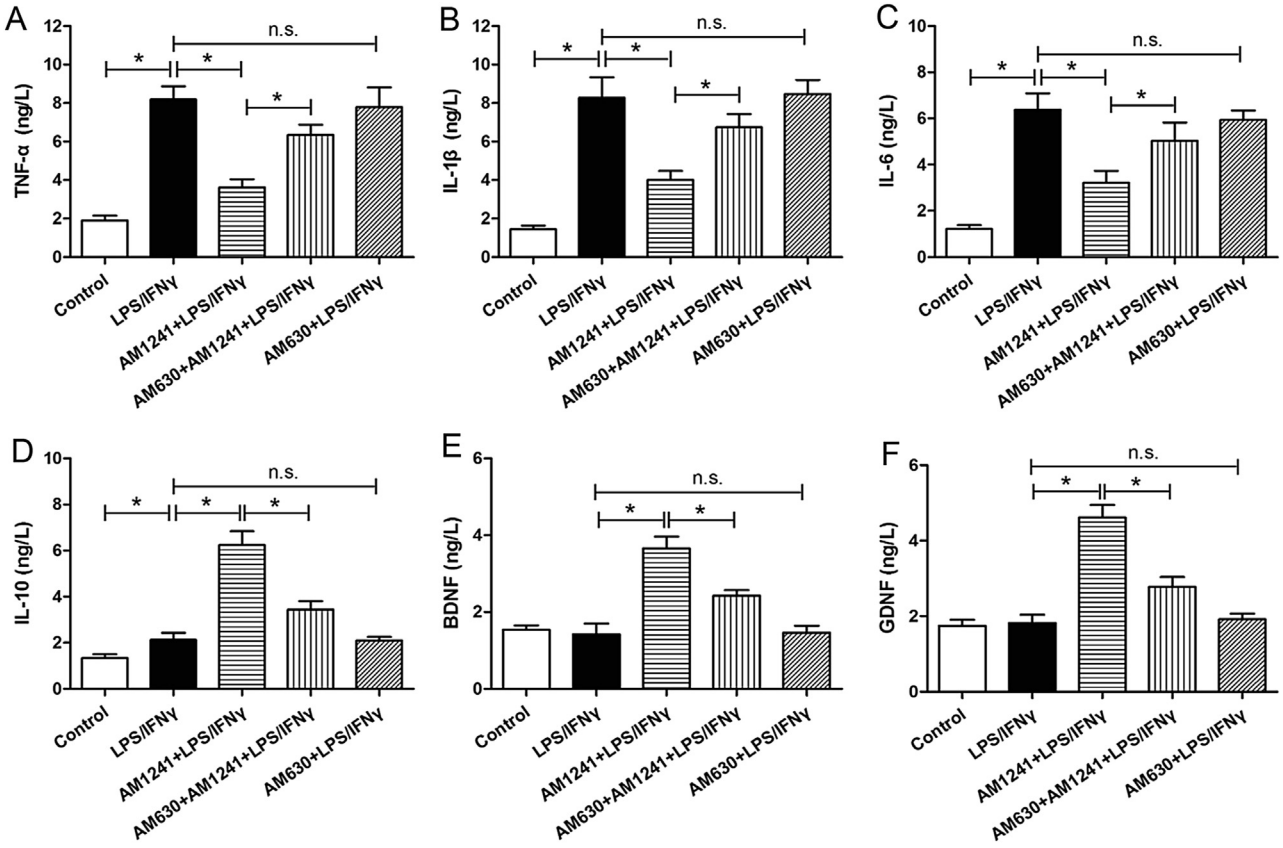

Fig. 3. Effects of AM1241 on the releases of pro-inflammatory, anti-inflammatory and neurotrophic factors from N9 microglia. The mouse N9 microglial cells were pretreated with or without 5 μM AM1241 in the presence or absence of 10 μM CB2 antagonist AM630. They were then exposed to 10 ng/ml LPS plus 10 U/ml IFNγ for 24 h at 1 h after AM1241 pretreatment. The levels of TNF-α (A), IL-1β (B), IL-6 (C), IL-10 (D), BDNF (E) and GDNF (F) in the supernatants were measured by ELISA assay (n = 8). Data are means ± SD. *: $P < 0.05$; n.s.: no significance.

3.4. The benifits of AM1241 pretreatment were significantly abolished by protein kinase C inhibitor

We also investigated the role of PKC in AM1241-induced anti-inflammation in microglia exposed to LPS plus IFNγ. AM1241 pretreatment attenuated iNOS expression, TNF-α and IL-1β release and increased IL-10 secretion in microglia exposed to LPS plus IFNγ (Fig. 4, $P < 0.05$). The benefits of AM1241 pretreatment were significantly reversed by 10 μM chelerythrine (Che), a PKC inhibitor ($P < 0.05$), suggesting PKC may be involved in the AM1241-induced anti-inflammation in microglia exposed to LPS plus IFNγ.

4. Discussion

In the present study, we found that pretreatment with CB2 receptor agonist AM1241 alleviated microglial activation induced by LPS plus IFNγ, decreased the expression of M1 marker iNOS and increased M2 marker Arg-1 expression in N9 microglia exposed to LPS plus IFNγ. In addition, AM1241 attenuated the releases of pro-inflammatory factors, enhanced the releases of anti-inflammatory and neurotrophic factors from microglia exposed to LPS plus IFNγ. These AM1241 pretreatment-induced benefits were significantly abolished in the presence of selective CB2 receptor antagonist AM630 or PKC inhibitor chelerythrine. These findings suggest that activation of CB2 receptor reduces microglial activation by shifting microglial M1 to M2 state, and PKC may participate in the benefits of AM1241 pretreatment.

Inflammatory reaction is involved in the pathophysiological process of many diseases in the CNS including brain trauma, stroke and neurodegenerative diseases [17,18]. Microglial cells, the resident macrophages in brain, play a key role in regulating cerebral inflammatory reactions [18]. In normal condition, microglial cells are quiescent. However, several agents can activate microglia, such as virus and β-amyloid (Aβ), to produce iNOS and pro-inflammatory factors which can induce neuron death [19]. In the present study, we used LPS plus IFNγ to activate mouse N9 microglia. LPS is a constituent of the outer membrane of gram-negative bacteria and has been used widely to induce experimental inflammatory reactions [20]. IFNγ can be used to enhance these effects of LPS [21]. The combination of the two agents is powerful to activate microglia into M1 state [22].

In the CNS, unlike other cells, microglial cells are the primary responding cells against pathogen infection [23]. Like macrophage, microglia polarize to M1 state by stimulation with LPS plus IFNγ and produce reactive oxygen species (ROS) and pro-inflammatory factors, such as TNF-α, IL-1β, IL-6 [23]. In contrast, several studies showed that IL-4 and IL-13 can up-regulate the expressions of Arg-1, Ym1, and CD36 in activated microglia and decrease the level of TNF-α in the CNS of mice [24]. So, IL-4 and IL-13 can induce microglia to polarize to M2 state, which express M2 markers, anti-inflammatory and neurotrophic factors, such as IL-10, BDNF and GDNF [23,25]. Therefore, it is possible that inducing the shift of microglial M1 to M2 state plays an important role in alleviating neuroinflammation in the CNS. The mouse N9 microglial cell line used in this study, like primary microglia, can be polarized into M1 or M2 state and secrete the markers of

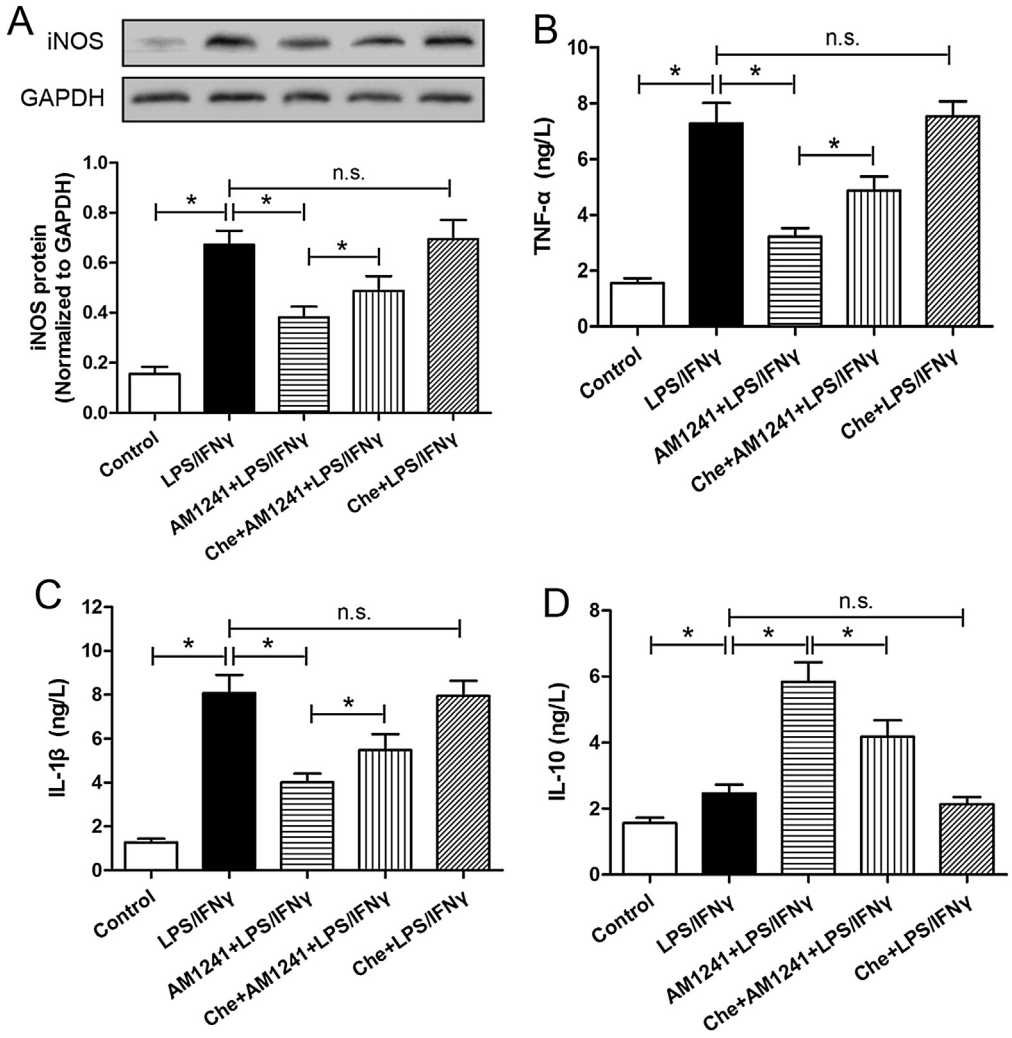

Fig. 4. The anti-inflammatory effects of AM1241 pretreatment were significantly abolished by PKC inhibitor in N9 microglia. The mouse N9 microglial cells were pretreated with or without 5 μM AM1241 in the presence or absence of 10 μM chelerythrine, a PKC inhibitor. They were then exposed to 10 ng/ml LPS plus 10 U/ml IFNγ for 24 h at 1 h after AM1241 pretreatment. (A) The expression of iNOS was analyzed by Western blot analysis (n = 6). The levels of TNF-α (B), IL-1β (C) and IL-10 (D) in the supernatants were measured by ELISA assay (n = 8). Data are means ± SD. *: $P < 0.05$; n.s.: no significance.

microglial M1 and M2 states, such as iNOS, TNF-α, IL-1β and Arg-1, in the presence of stimulus [26,27]. Thus, we used N9 microglia to study microglial M1 and M2 states in this investigation.

It is well known that CB2 receptor activation can produce anti-inflammatory effects [28]. Especially, activation of CB2 receptor during neuroinflammation diminishes the production of NO and TNF-α in primary microglia [29,30]. In this study, CB2 agonist AM1241 pretreatment attenuated the expression of M1 marker iNOS and the levels of pro-inflammatory factors including TNF-α, IL-1β and IL-6; increased the expression of M2 marker Arg-1 and the levels of anti-inflammatory and neurotrophic factors including IL-10, BDNF and GDNF in N9 microglia exposed to LPS plus IFNγ. The benefits of AM1241 were significantly reversed in the presence of CB2 receptor antagonist AM630. In addition, several studies have showed that various isoforms of PKC could be activated in microglia by LPS, Aβ or INFγ [31–33], and some researchers found that inhalable anesthetic isofurane-induced anti-inflammation in microglia and macrophages was mediated by PKC [13,34]. Moreover, we have ever reported that epsilon PKC mediated electroacupuncture-induced neuroprotection by activating cannabinoid CB1 receptor [14]. Therefore, we inferred that PKC may participate in the CB2 agonist AM1241-induced anti-inflammation. In the present study, we found that the benefits of AM1241 pretreatment were partially reversed by chelerythrine, a PKC inhibitor, indicating PKC may be involved in the AM1241-induced anti-inflammation in N9 microglial cells.

In this study, we found that CB2 agonist pretreatment attenuated microglial activation by shifting microglial M1 to M2 state, and induced anti-inflammatory and neurotrophic effects. Our investigation suggests that CB2 receptor in microglia may be a potential novel target for the treatment of neuroinflammatory disorders.

In summary, this study has shown that CB2 agonist AM1241 pretreatment inhibits microglial activation by inducing the shift of M1 to M2 state in the presence of LPS plus IFNγ, and the AM1241 pretreatment-induced benefits may be mediated by PKC.

Conflict of interest

All authors of this manuscript declare no conflict of interest.

Acknowledgments

This study was supported by the Changjiang Scholars and Innovative Research Team in University (No. IRT1053).

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.073.

References

[1] S.D. Skaper, P. Giusti, L. Facci, Microglia and mast cells: two tracks on the road to neuroinflammation, FASEB J. 26 (2012) 3103–3117.
[2] E. Czirr, T. Wyss-Coray, The immunology of neurodegeneration, J. Clin. Invest. 122 (2012) 1156–1163.
[3] J. Hur, P. Lee, M.J. Kim, Y. Kim, Y.W. Cho, Ischemia-activated microglia induces neuronal injury via activation of gp91phox NADPH oxidase, Biochem. Biophys. Res. Commun. 391 (2010) 1526–1530.
[4] T. Deierborg, L. Roybon, A.R. Inacio, J. Pesic, P. Brundin, Brain injury activates microglia that induce neural stem cell proliferation ex vivo and promote differentiation of neurosphere-derived cells into neurons and oligodendrocytes, Neuroscience 171 (2010) 1386–1396.
[5] E. Polazzi, B. Monti, Microglia and neuroprotection: from in vitro studies to therapeutic applications, Prog. Neurobiol. 92 (2010) 293–315.
[6] S.K. Biswas, M. Chittezhath, I.N. Shalova, J.Y. Lim, Macrophage polarization and plasticity in health and disease, Immunol. Res. 53 (2012) 11–24.
[7] E. Jang, S. Lee, J.H. Kim, J.H. Kim, J.W. Seo, W.H. Lee, K. Mori, K. Nakao, K. Suk, Secreted protein lipocalin-2 promotes microglial M1 polarization, FASEB J. 27 (2013) 1176–1190.
[8] G.A. Cabral, L. Griffin-Thomas, Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation, Expert. Rev. Mol. Med. 11 (2009) e3.
[9] J.P. Gong, E.S. Onaivi, H. Ishiguro, Q.R. Liu, P.A. Tagliaferro, A. Brusco, G.R. Uhl, Cannabinoid CB2 receptors: immunohistochemical localization in rat brain, Brain Res. 1071 (2006) 10–23.
[10] E.S. Onaivi, H. Ishiguro, J.P. Gong, S. Patel, A. Perchuk, P.A. Meozzi, L. Myers, Z. Mora, P. Tagliaferro, E. Gardner, A. Brusco, B.E. Akinshola, Q.R. Liu, B. Hope, S. Iwasaki, T. Arinami, L. Teasenfitz, G.R. Uhl, Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain, Ann. N. Y. Acad. Sci. 1074 (2006) 514–536.
[11] J. Fernandez-Ruiz, J. Romero, G. Velasco, R.M. Tolon, J.A. Ramos, M. Guzman, Cannabinoid CB2 receptor: a new target for controlling neural cell survival? Trends Pharmacol. Sci. 28 (2007) 39–45.
[12] K.D. Patel, J.S. Davison, Q.J. Pittman, K.A. Sharkey, Cannabinoid CB(2) receptors in health and disease, Curr. Med. Chem. 17 (2010) 1393–1410.
[13] X. Xu, J.A. Kim, Z. Zuo, Isoflurane preconditioning reduces mouse microglial activation and injury induced by lipopolysaccharide and interferon-gamma, Neuroscience 154 (2008) 1002–1008.
[14] Q. Wang, X. Li, Y. Chen, F. Wang, Q. Yang, S. Chen, Y. Min, X. Li, L. Xiong, Activation of epsilon protein kinase C-mediated anti-apoptosis is involved in rapid tolerance induced by electroacupuncture pretreatment through cannabinoid receptor type 1, Stroke 42 (2011) 389–396.
[15] L.C. Chang, L.T. Tsao, C.S. Chang, C.J. Chen, L.J. Huang, S.C. Kuo, R.H. Lin, J.P. Wang, Inhibition of nitric oxide production by the carbazole compound LCY-2-CHO via blockade of activator protein-1 and CCAAT/enhancer-binding protein activation in microglia, Biochem. Pharmacol. 76 (2008) 507–519.
[16] G. Benard, F. Massa, N. Puente, J. Lourenco, L. Bellocchio, E. Soria-Gomez, I. Matias, A. Delamarre, M. Metna-Laurent, A. Cannich, E. Hebert-Chatelain, C. Mulle, S. Ortega-Gutierrez, M. Martin-Fontecha, M. Klugmann, S. Guggenhuber, B. Lutz, J. Gertsch, F. Chaouloff, M.L. Lopez-Rodriguez, P. Grandes, R. Rossignol, G. Marsicano, Mitochondrial CB(1) receptors regulate neuronal energy metabolism, Nat. Neurosci. 15 (2012) 558–564.
[17] M. Eddleston, L. Mucke, Molecular profile of reactive astrocytes–implications for their role in neurologic disease, Neuroscience 54 (1993) 15–36.
[18] G.W. Kreutzberg, Microglia: a sensor for pathological events in the CNS, Trends Neurosci. 19 (1996) 312–318.
[19] G.C. Brown, A. Bal-Price, Inflammatory neurodegeneration mediated by nitric oxide, glutamate, and mitochondria, Mol. Neurobiol. 27 (2003) 325–355.
[20] J. Reutershan, D. Chang, J.K. Hayes, K. Ley, Protective effects of isoflurane pretreatment in endotoxin-induced lung injury, Anesthesiology 104 (2006) 511–517.
[21] J.C. Zhuang, G.N. Wogan, Growth and viability of macrophages continuously stimulated to produce nitric oxide, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 11875–11880.
[22] W.K. Jung, D.Y. Lee, C. Park, Y.H. Choi, I. Choi, S.G. Park, S.K. Seo, S.W. Lee, S.S. Yea, S.C. Ahn, C.M. Lee, W.S. Park, J.H. Ko, I.W. Choi, Cilostazol is anti-inflammatory in BV2 microglial cells by inactivating nuclear factor-kappaB and inhibiting mitogen-activated protein kinases, Br. J. Pharmacol. 159 (2010) 1274–1285.
[23] K. Saijo, C.K. Glass, Microglial cell origin and phenotypes in health and disease, Nat. Rev. Immunol. 11 (2011) 775–787.
[24] K. Kawahara, M. Suenobu, A. Yoshida, K. Koga, A. Hyodo, H. Ohtsuka, A. Kuniyasu, N. Tamamaki, Y. Sugimoto, H. Nakayama, Intracerebral microinjection of interleukin-4/interleukin-13 reduces beta-amyloid accumulation in the ipsilateral side and improves cognitive deficits in young amyloid precursor protein 23 mice, Neuroscience 207 (2012) 243–260.
[25] J. Mikita, N. Dubourdieu-Cassagno, M.S. Deloire, A. Vekris, M. Biran, G. Raffard, B. Brochet, M.H. Canron, J.M. Franconi, C. Boiziau, K.G. Petry, Altered M1/M2 activation patterns of monocytes in severe relapsing experimental rat model of multiple sclerosis. Amelioration of clinical status by M2 activated monocyte administration, Mult. Scler. 17 (2011) 2–15.
[26] Y. Tang, T. Li, J. Li, J. Yang, H. Liu, X. Zhang, W. Le, Jmjd3 is essential for the epigenetic modulation of microglia phenotypes in the immune pathogenesis of Parkinson's disease, Cell Death Differ. 21 (2014) 369–380.
[27] H. Liu, M. Zheng, Y. Du, L. Wang, F. Kuang, H. Qin, B. Zhang, H. Han, N9 microglial cells polarized by LPS and IL4 show differential responses to secondary environmental stimuli, Cell. Immunol. 278 (2012) 84–90.
[28] F. Correa, M. Hernangomez, L. Mestre, F. Loria, A. Spagnolo, F. Docagne, V. Di Marzo, C. Guaza, Anandamide enhances IL-10 production in activated microglia by targeting CB(2) receptors: roles of ERK1/2, JNK, and NF-kappaB, Glia 58 (2010) 135–147.
[29] S. Merighi, S. Gessi, K. Varani, C. Simioni, D. Fazzi, P. Mirandola, P.A. Borea, Cannabinoid CB(2) receptors modulate ERK-1/2 kinase signalling and NO release in microglial cells stimulated with bacterial lipopolysaccharide, Br. J. Pharmacol. 165 (2012) 1773–1788.
[30] C. Benito, R.M. Tolon, M.R. Pazos, E. Nunez, A.I. Castillo, J. Romero, Cannabinoid CB2 receptors in human brain inflammation, Br. J. Pharmacol. 153 (2008) 277–285.
[31] S. Shen, S. Yu, J. Binek, M. Chalimoniuk, X. Zhang, S.C. Lo, M. Hannink, J. Wu, K. Fritsche, R. Donato, G.Y. Sun, Distinct signaling pathways for induction of type II NOS by IFNgamma and LPS in BV-2 microglial cells, Neurochem. Int. 47 (2005) 298–307.
[32] C.K. Combs, D.E. Johnson, S.B. Cannady, T.M. Lehman, G.E. Landreth, Identification of microglial signal transduction pathways mediating a neurotoxic response to amyloidogenic fragments of beta-amyloid and prion proteins, J. Neurosci. 19 (1999) 928–939.
[33] J. Wen, R. Ribeiro, Y. Zhang, Specific PKC isoforms regulate LPS-stimulated iNOS induction in murine microglial cells, J. Neuroinflam. 8 (2011) 38.
[34] X. Xu, J. Feng, Z. Zuo, Isoflurane preconditioning reduces the rat NR8383 macrophage injury induced by lipopolysaccharide and interferon gamma, Anesthesiology 108 (2008) 643–650.