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ORIGINAL RESEARCH

Dexmedetomidine Attenuates Neuroinflammation In LPS-Stimulated BV2 Microglia Cells Through Upregulation Of miR-340

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Background: Dexmedetomidine (Dex) was reported to exhibit anti-inflammatory effect in the nervous system. However, the mechanism by which Dex exhibits anti-inflammation effects on LPS-stimulated BV2 microglia cells remains unclear. Thus, this study aimed to investigate the role of Dex in LPS-stimulated BV2 cells.

Methods: The BV2 cells were stimulated by lipopolysaccharides (LPS). BV2 cells were infected with short-hairpin RNAs targeting NF-κB (NF-κB-shRNAs) and NF-κB overexpression lentivirus, respectively. In addition, miR-340 mimics or miR-340 inhibitor was transfected into BV2 cells, respectively. Meanwhile, the dual-luciferase reporter system assay was used to explore the interaction of miR-340 and NF-κB in BV2 cells. CCK-8 was used to detect the viability of BV2 cells. In addition, Western blotting was used to detect the level of NF-κB in LPS-stimulated BV2 cells. The levels of TNF-α, IL-6, IL-1β, IL-2, IL-12, IL-10 and MCP-1 in LPS-stimulated BV2 cells were measured with ELISA.

Results: The level of miR-340 was significantly upregulated in Dex-treated BV2 cells. Meanwhile, the level of NF-κB was significantly increased in BV2 cells following infection with lenti-NF-κB, which was markedly reversed by Dex. LPS markedly increased the expression of NF-κB and proinflammatory cytokines in BV2 cells, which were reversed in the presence of Dex. Moreover, miR-340 mimics enhanced the anti-inflammatory effects of Dex in LPS-stimulated BV2 cells via inhibiting NF-κB and proinflammatory cytokines. Furthermore, Dex obviously inhibited LPS-induced phagocytosis in BV2 cells.

Conclusion: Taken together, our results suggested that Dex might exert anti-inflammatory effects in LPS-stimulated BV2 cells via upregulation of miR-340. Therefore, Dex might serve as a potential agent for the treatment of neuroinflammation.

Keywords: NF-κB, dexmedetomidine, BV2 microglia cells, postoperative cognitive dysfunction

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Introduction

Postoperative cognitive dysfunction (POCD), a common clinical complication, was generated in patients who suffer the anesthesia and hypoxic intervals during surgery.[1] The morbidity of POCD was 16–40% at 7 days after surgery in middle-aged and elderly patients.[2] POCD could influence information processing speed, memory and executive function in patients, especially in middle-aged and elderly patients.[3,4] In addition, POCD severely reduced the quality of life and increased death rate in patients.[5] Thus, it is imminently for us to take preventive measures to decrease the incidence.

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It has been reported that POCD was related to an elevated level of neuroinflammation.[3] Neuroinflammation induced by surgical trauma leads to POCD.[6] Microglia, a resident macrophage in the central nervous system, plays an important role in regulating immune response and neuronal homeostasis.[7] Microglia was activated and then secreted several pro-inflammatory cytokines during the occurrence of inflammation.[7] In addition, nuclear factor (NF)-κB is a transcription factor, which plays an important role in the inflammatory response of microglia cells.[8] NF-κB could regulate multiple inflammatory cytokines including IL-2, IL-6, IL-1β and TNF-α.[9,10] Zhang et al indicated that surgery could increase the levels of NF-κB, IL-6 and IL-1β in rats.[11] However, inhibition of NF-κB could attenuate neuroinflammation and succedent POCD.[12] Previous study indicated that suppression of NF-κB signaling pathway could alleviate POCD after nerve anesthesia.[13]

Dexmedetomidine (Dex) is a selective α2-adrenergic agonist, which exhibits a neuroprotective role.[14] Dex was used as an auxiliary sedative or an agent from mechanical ventilation.[15] Previous study found that Dex could decrease the duration of mechanical ventilation and the morbidity of POCD.[16,17] In addition, Dex could alleviate POCD in rats via regulating cAMP signaling pathway.[18] Moreover, Li et al found that Dex decreased the production of inflammatory factors and reduced postoperative recurrent rate in patients with POCD.[19]

In spite of many reports indicated that Dex exerted a protective effect of the nervous system via inhibiting inflammatory response,[20,21] little is known about the relationship of Dex and NF-κB pathway. In addition, previous study reported that POCD was associated with microRNAs (miRNAs).[22] Therefore, the present study aimed to investigate the neuroprotective effect of Dex in LPS-stimulated BV2 cells from the perspective of miRNAs.

Materials And Methods

Cell Culture

Due to the low cell number and time-consuming techniques needed to cultivate primary microglia cultures, the immortalized microglia BV2 cell line has been used extensively in the study associated with neurodegenerative disorders.[23] We used lipopolysaccharide (LPS) to simulate BV2 microglia cells to mimic the inflammatory environment in the brain.[24] The BV2 cell line was purchased from Conservation Genetics CAS Kunming Cell Bank (Kunming, China). BV2 cells were cultured in DMEM medium with 10% fetal bovine serum (FBS, Thermo Fisher Scientific, Waltham, MA, USA) and penicillin-streptomycin (100 μg/mL, Thermo Fisher Scientific) at 37°C with 5% CO_2. Dex was obtained from Sigma-Aldrich (St. Louis, MO, USA).

CCK-8 Assay Of Cell Viability

Cell viability was measured by cell counting kit-8 (CCK-8, Sigma) according to the specification. BV2 cells were seed into 96-well plates at a density of 5000 cells per well overnight at 37°C. Then, cells were treated with Dex (0, 4, 8, 12, 16, 20 or 25 μg/mL) for 48 hrs at 37°C. Later on, after incubation with CCK-8 reagent (10 μL) for 2 hrs, microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) was used to measure the absorbance of BV2 cells at a wavelength of 450 nm.

Lentiviral Construction And Cell Infection

The NF-κB sequence was synthesized by GenePharma (Shanghai, China). The NF-κB-shRNA1, NF-κB-shRNA2 and NF-κB-shRNA3 plasmids were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and then sub-cloned into the pWPXL (lentiviral expression vector). 293T cells were co-transfected with pWPXL-NF-κB, NF-κB-shRNAs plasmids or pSuper-puro-GFP vector, respectively. Forty-eight hours later, the supernatant of cells was collected.

BV2 cells were infected with 1×10^7 NF-κB lentivirus or NF-κB lentivirus-shRNAs transducing units containing 5 μg/mL polybrene (Sigma) for 48 hrs, respectively. For stably infected cells, BV2 cells were treated with puromycin (2.5 μg/mL, Invitrogen) for 72 hrs. Real-time qPCR and Western blotting assays were used to measure the expression of NF-κB in the BV2 cells.

Cell Transfection

BV2 cells (4×10^5 cells per well) were seed onto 6-well plates overnight to reach 70% confluence for transfection. Then, miR-340 mimic or miR-34 inhibitor was transfected into cells for 24 hrs using Lipofectamine 2000 (Thermo Fisher Scientific) according to the manufacturer's instructions. After that, transfected cells were treated with 20 μg/mL Dex for another 24 hrs at 37°C.

Real-Time qPCR

Trizol kit (Thermo Fisher Scientific) was used to obtain the total RNA according to the manufacturer's procedure. Then, cDNA was synthesized by using a cDNA synthesis kit (Thermo Fisher Scientific). After that, SYBR premix Ex Taq II kit (TaKaRa, Dalian, China) was used to perform the

RT-qPCR reaction on an ABI 7900HT instrument (ABI, NY, USA) according to the manufacturer's procedure. The qPCR reaction conditions were as follows: 94°C for 3 mins followed by 45 cycles of 94°C for 45 s, 56°C for 30 s, finally, 72°C for 45 s. NF-κB: F: 5′-ACGATCTGTTTCCCCTCATCT-3′; R: 5′-TGCTTCTCTCCCCAGGAATA-3′, miR-572: F: 5′-GCCAGATCTCTGAGGAAAGCAGGAGGAGG-3′; R: 5′-GCCGAATTCTCGGCACAAATCTTCAGAGC-3′, miR-155: F: 5′-GTAACCCGTTGAACCCCATT-3′; R: 5′-CCATCCAATCGGTAGTAGCG-3′, miR-340: F: 5′-GTGGGGTTATAAAGCAATGAGA-3′; R: 5′-GTGCAGGGTCCGAGGT-3′, GAPDH: F: 5′-CTGGGGACGACATGGAGAAAA-3′; R: 5′-AAGGAAGGCTGGAAGAGTGC-3′. U6: F: 5′-CTCGCTTCGGCAGCACAT-3′; R: 5′-AACGCTTCACGAATTTGCGT-3′. The primers were obtained from GenePharma (Shanghai, China). The $2^{-\Delta\Delta Ct}$ method was used to analyze the data (Supplementary figure 1).[25] The level of NF-κB was normalized by internal control GAPDH.[26,27] The levels of miR-572, miR-155 and miR-340 were normalized to internal control U6.

Western Blot Analysis

Total proteins were qualified by using a BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein (30 μg per lane) were separated on 10% SDS-PAGE and then transferred onto PVDF membranes (Thermo Fisher Scientific). The membranes were blocked with 5% skim milk for 1 hr at room temperature and then incubated with the primary antibody overnight at 4°C. Primary antibodies: anti-NF-κB (Abcam, 1:1000) and anti-GAPDH (Abcam, 1:1000). After that, the membranes were incubated with anti-rabbit IgG secondary antibodies for 50 mins at room temperature. Later on, the blots were visualized by ECL reagent and then captured using the ChemiDoc™XRS+ imaging system (Bio-Rad, Hercules, CA, USA).

Luciferase Reporter Assay

Reporter plasmids of wild-type (WT)-NF-κB and mutant (MT)-NF-κB were synthesized by GenePharma. BV2 cells were cultured to reach 70% confluence. The designed WT-NF-κB or MT-NF-κB and corresponding plasmids (miR-340 mimics or miR-340 inhibitor) were then co-transfected into BV2 cells for 48 hrs using Lipofectamine 2000. The luciferase report activity was measured with the Dual-Luciferase Reporter Assay System (Promega). The rellina luciferase activity was normalized to control group.

Cytokine Analysis By ELISA

BV2 cells (4×10^5 cells per well) were seeded onto 6-well plates overnight at 37°C. The levels of TNF-α, IL-6, IL-1β, IL-2, IL-12, IL-10 and MCP-1 in the supernatant were measured with ELISA kits (KAINOS laboratories, Japan) in accordance with the manufacturer's instructions.

Phagocytosis Assay

BV2 cells were incubated with FITC-labeled latex beads (Sigma Aldrich, St. Louis, MO, USA) for 1 hr at 37°C. Then, the cells were washed in PBS and analyzed using the Cell Quest Software (BD Biosciences, San Jose, CA) on a FACS Calibur flow cytometer (BD Biosciences).

Statistical Analysis

Each group was performed at least three independent experiments and all data were expressed as the mean ± standard deviation (SD). SPSS v21.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism v5.0 (GraphPad Software, Inc., La Jolla, CA, USA) were used to analyze the experimental data. The comparison between two groups was analyzed by Student's *t*-test. The comparisons among multiple groups were made with one-way analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

The Effect Of Dex On The Viability Of BV2 Cells

CCK-8 assay was applied to determine the effect of Dex on the viability of BV2 cells. As shown in Figure 1, 25 μg/mL Dex significantly decreased the viability of BV2 cells. Meanwhile, 20 μg/mL Dex had a very limited effect on the proliferation of BV2 cells. Therefore, 20 μg/mL Dex was utilized in the following experiments.

Dex Down-Regulated The Expression Of NF-κB In BV2 Cells

It has been reported that NF-κB plays an important role in neuroinflammation and POCD.[28] In order to investigate the role of NF-κB in BV2 cells, cells were infected with lentivirus-NF-κB (lenti-NF-κB) or NF-κB-shRNAs. As illustrated in Figure 2A, BV2 cells were effectively infected with lenti-NF-κB after 48 hrs of incubation. In addition, RT-qPCR and Western blotting indicated that the level of NF-κB was significantly upregulated following transfection with lenti-NF-κB, compared with control

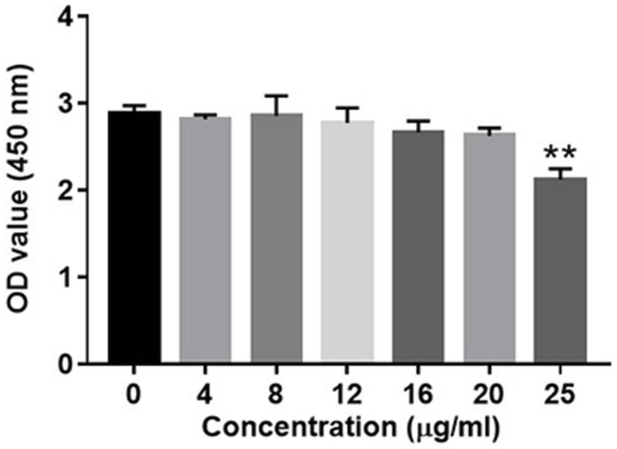

Figure 1 The effects of Dex on proliferation in BV2 cells. CCK-8 assay was used to detect the viability of BV2 cells. BV2 cells were treated with Dex (0, 4, 8, 12, 16, 20 or 25 μg/mL) for 48 hrs. **P < 0.01 compared with control group.

group (Figure 2B–D). Moreover, the results indicated that the expression of NF-κB was decreased most significantly in BV2 cells after infection with NF-κB-shRNA3 compared with the control group and other NF-κB-shRNAs (Figure 2E–H). Thus, NF-κB shRNA3 was utilized in the following experiments.

To further investigate the association between the NF-κB gene and Dex in BV2 cells, Western blot was used. The result indicated the level of NF-κB was significantly increased following transfection with lenti-NF-κB, which was markedly reversed by Dex (Figure 2I and J). In addition, Dex notably decreased the expression of NF-κB in cells, which was further decreased following infection with NF-κB-shRNA3 (Figure 2I and J). These results indicated that Dex could down-regulate NF-κB gene expression in BV2 cells.

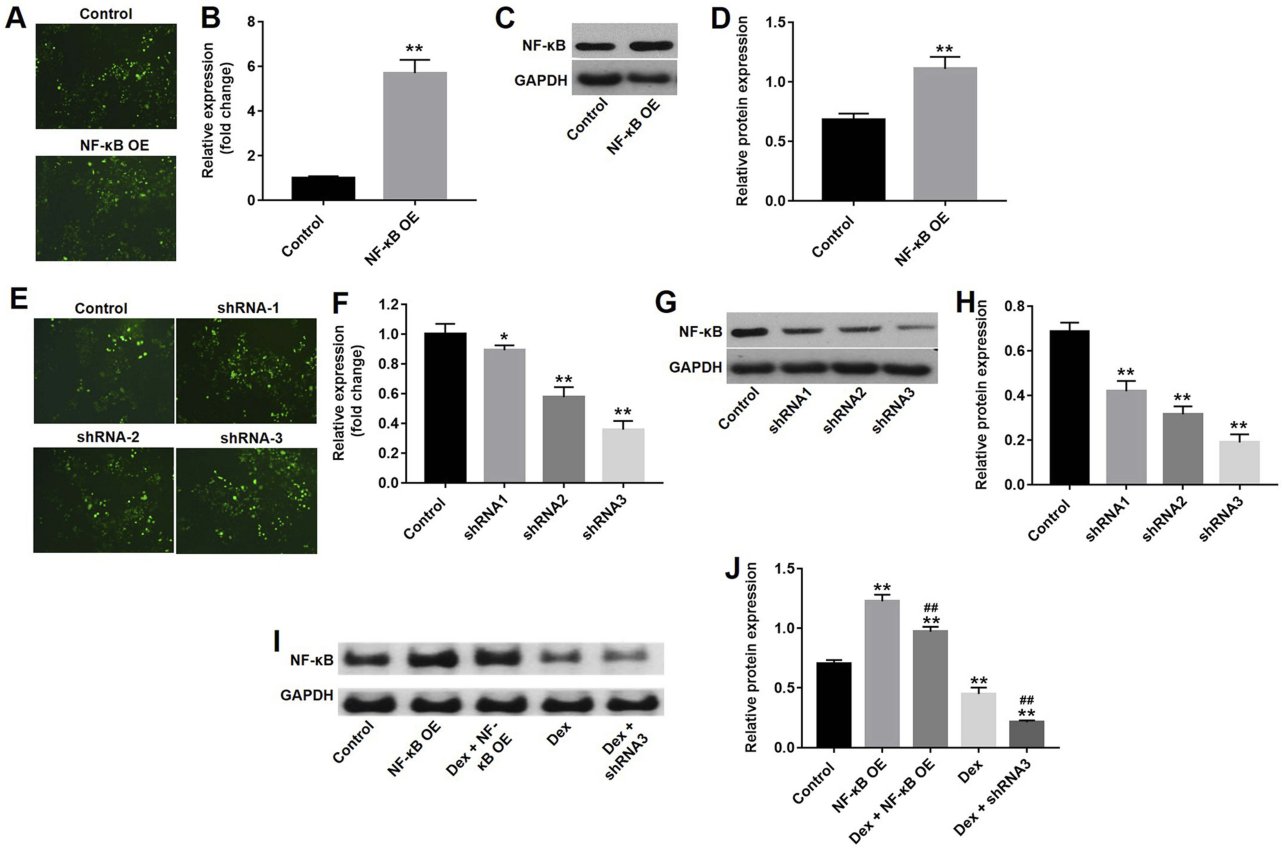

Figure 2 Dex down-regulated NF-κB gene expression in BV2 cells. (**A**) Lenti-control (control) and lenti-NF-κB (NF-κB-OE) were transfected into BV2 cells. Fluoresce microscope was used to observe the transfection efficacy of control-GFP and NF-κB-OE-GFP plasmids in BV2 cells (200× magnification). (**B**) RT-qPCR was used to detect the level of NF-κB in BV2 cells. (**C, D**) Western blot was used to detect the expression of NF-κB in BV2 cells. (**E**) Lenti-control (control), NF-κB-shRNA1, NF-κB-shRNA2, NF-κB-shRNA3 were transfected into BV2 cells. Fluoresce microscope was used to observe the transfection efficacy of control-GFP and NF-κB-shRNAs-GFP plasmids in BV2 cells (200× magnification). (**F**) RT-qPCR was used to detect the level of NF-κB in BV2 cells. (**G, H**) Western blot was used to detect the expression of NF-κB in BV2 cells. (**I, J**) BV2 cells were treated with 20 μg/mL DEX, and then lenti-NF-κB or NF-κB-shRNA3 were transfected into BV2 cells for 48 hrs simultaneously. Western blot was used to detect the expression of NF-κB in BV2 cells. *P < 0.05, **P < 0.01 compared with control group, ##P < 0.01 compared with NF-κB-OE or shRNA 3 group.

miR-340 Mimics Enhanced The Anti-Inflammatory Effect Of Dex In BV2 Cells Via Inhibiting NF-κB

Previous studies indicated that some miRNAs were associated with the occurrence of POCD, such as miR-572 and miRNA-155.[29,30] In addition, miR-340 was related to the activity of NF-κB.[31,32] Meanwhile, it has been shown that miR-340 could inhibit the progression of glioblastoma cells.[33] Therefore, we next explored the association between NF-κB gene and miRNAs in BV2 cells. As demonstrated in Figure 3A, the level of miR-340 was significantly upregulated in Dex-treated BV2 cells. In addition, the level of miR-340 was markedly increased in miR-340 mimics group, while its level was significantly decreased in the miR-340 inhibitor group (Figure 3B and C).

Next, as shown in Figure 3D and E, Dex significantly decreased the expression of NF-κB in BV2 cells, which were further markedly reduced following transfection with miR-340 mimics. However, Dex-induced NF-κB protein decrease in cells was markedly inhibited by miR-340 inhibitor (Figure 3D and E). In addition, the luciferase assay data indicated that the reduced luciferase activity was observed in BV2 cells following transfection with NF-κB-WT and miR-340 mimics, while the increased luciferase activity was observed in BV2 cells following transfection with NF-κB-WT and miR-340 inhibitor (Figure 3F). These data suggested that overexpression of miR-340 could enhance the anti-inflammatory effect of Dex in BV2 cells via inhibiting NF-κB.

Overexpression Of miR-340 Enhanced The Inhibitory Effect Of Dex on NF-κB Pathway-Associated Cytokines In BV2 Cells

Next, ELISA assay was used to detect the levels of NF-κB pathway-associated cytokines in BV2 cells. As shown in Figure 4A–E, NF-κB pathway-associated pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IL-2 and IL-12 were

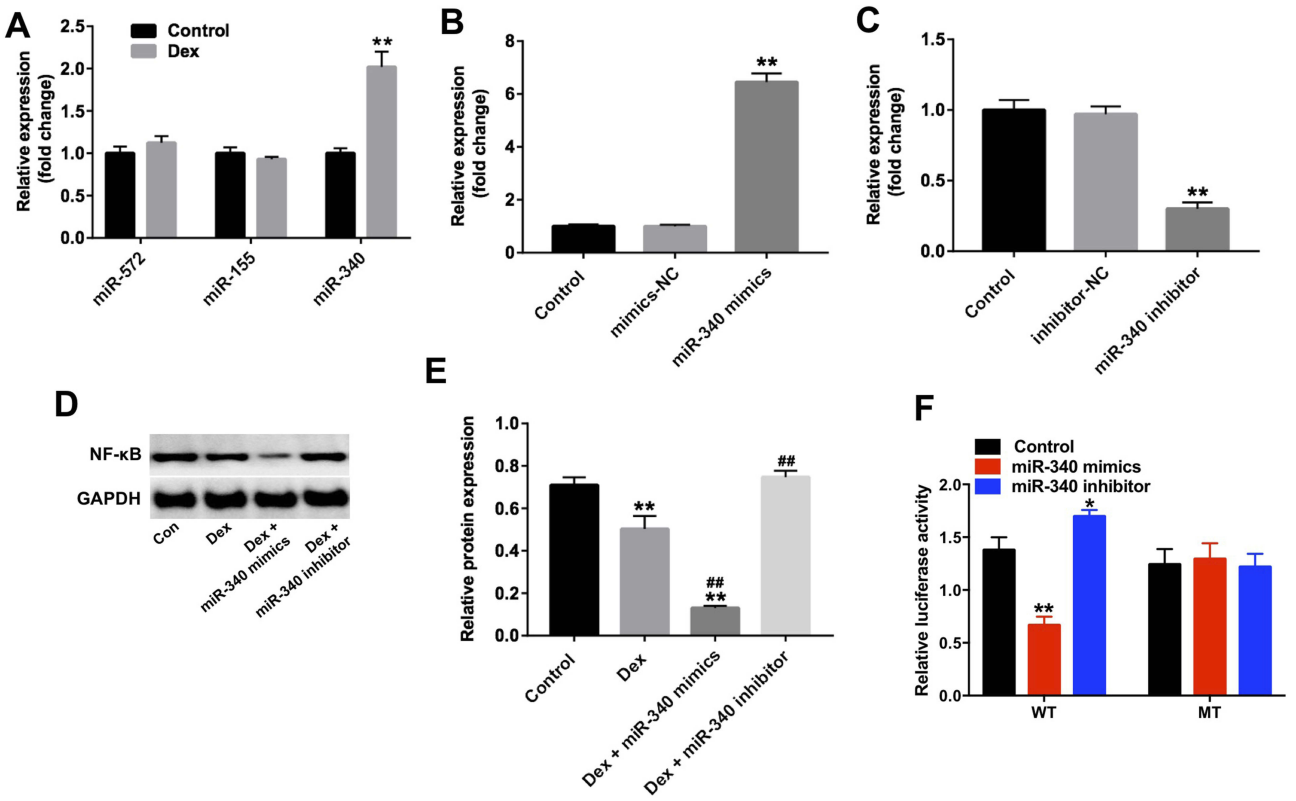

Figure 3 Overexpression of miR-340 enhanced the anti-inflammatory effect of Dex in BV2 cells via inhibiting NF-κB. (**A**) BV2 cells were treated with 20 μg/mL Dex for 48 hrs. Relative expression of miR-572, miR-155 and miR-340 in BV2 cells were detected by RT-qPCR. (**B**) BV2 cells were transfected with 10 nM miR-340 mimics for 48 hrs. Relative expression of miR-340 in BV2 cells were detected by RT-qPCR. (**C**) BV2 cells were transfected with 10 nM miR-340 inhibitor for 48 hrs. Relative expressions of miR-340 in BV2 cells were detected by RT-qPCR. (**D, E**) MiR-340 mimic or miR-34 inhibitor was transfected into BV2 cells, respectively. After 24 hrs, transfected cells were treated with 20 μg/mL Dex for another 24 hrs. Western blot was used to detect the expression of NF-κB in BV2 cells. (**F**) WT-NF-κB or MT-NF-κB was co-transfected with corresponding plasmids (miR-340 mimics or miR-340 inhibitor) in BV2 cells, respectively. The luciferase activity was measured by using the dual-luciferase reporter assay. *P < 0.05, **P < 0.01 compared with control group, ##P < 0.01 compared with Dex group.

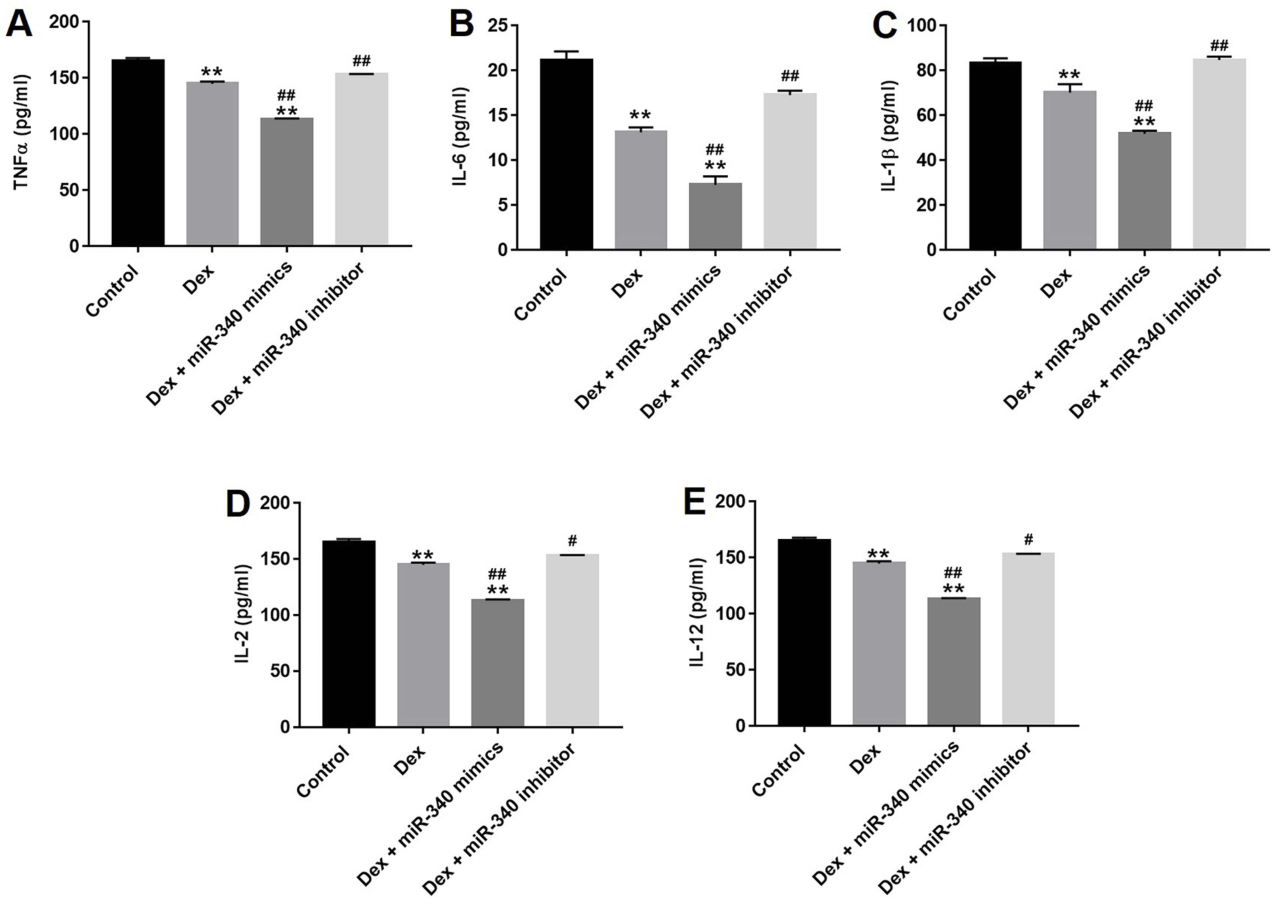

Figure 4 Overexpression of miR-340 enhanced the anti-inflammatory effect of Dex in BV2 cells via inhibiting NF-κB pathway-associated cytokines. miR-340 mimic and miR-34 inhibitors were transfected into BV2 cells, respectively. After 24 hrs, transfected cells were treated with 20 μg/mL Dex for another 24 hrs. (**A**) The level of TNF-α in the culture media was measured with ELISA. (**B**) The level of IL-6 in the culture media was measured with ELISA. (**C**) The level of IL-1β in the culture media was measured with ELISA. (**D**) The level of IL-2 in the culture media was measured with ELISA. (**E**) The level of IL-12 in the culture media was measured with ELISA. **P < 0.01 compared with control group, #P < 0.05, ##P < 0.01 compared with Dex group.

significantly decreased in Dex-treated BV2 cells, which were further reduced following transfection with miR-340 mimics. However, Dex-induced pro-inflammatory cytokine decreases were markedly reversed following transfection with miR-340 inhibitor. These data suggested that over-expression of miR-340 enhanced the inhibitory effect of Dex on NF-κB pathway-associated cytokines in BV2 cells.

Overexpression Of miR-340 Enhanced The Anti-Inflammatory Effect Of Dex In LPS-Stimulated BV2 Cells

Next, to further investigate the effects of Dex on LPS-induced neuroinflammation, Western blot and ELISA assays were performed. As shown in Figure 5A–H, LPS significantly increased the expression of NF-κB and the production of pro-inflammatory cytokines TNF-α, IL-6, IL-1β, IL-2, IL-12 and MCP-1 in BV2 cells. However, the expression of NF-κB and the levels of pro-inflammatory cytokines were markedly decreased in the presence of Dex, which was further enhanced following transfection with miR-340 mimics. Meanwhile, the production of anti-inflammatory cytokine IL-10 was markedly upregulated in the presence of Dex on LPS-stimulated BV2 cells, which was further increased following transfection with miR-340 mimics (Figure 5I). These data indicated that over-expression of miR-340 could enhance the anti-inflammatory effect of Dex in LPS-stimulated BV2 cells.

Dex Inhibited LPS-Induced Phagocytosis In BV2 Cells

Further, we examined whether Dex could regulate phagocytosis in BV2 cells. The experiment was performed using fluorescent latex beads in order to induce detectable engulfment by BV2 cells. As shown in Figure 6A and B, the number of phagocytic cells was obviously increased in

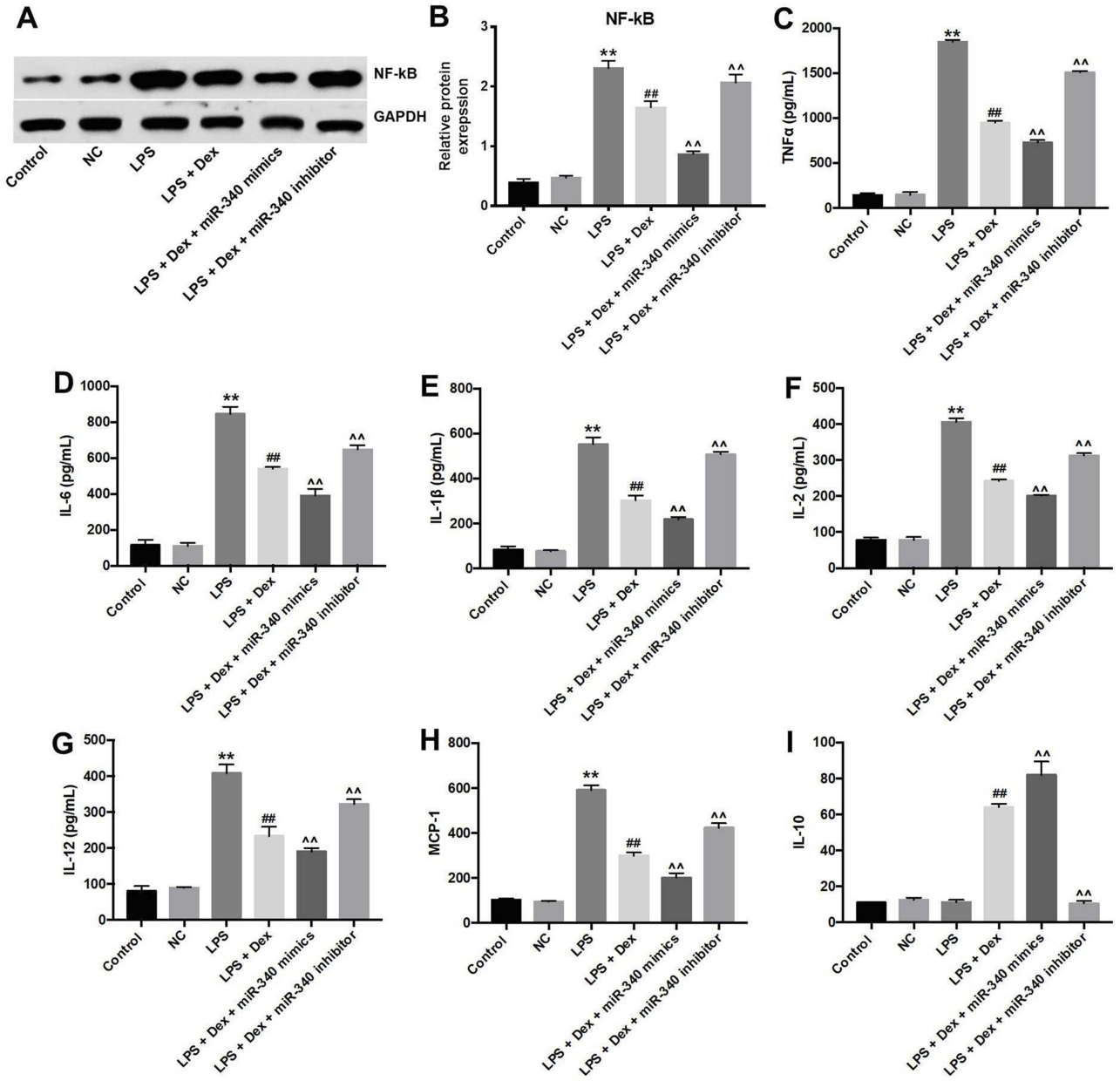

Figure 5 Overexpression of miR-340 enhanced the anti-inflammatory effect of Dex in LPS-stimulated BV2 cells. BV2 cells were transfected with miR-340 mimics or miR-340 inhibitor for 24 hrs and then incubated with Dex for 30 mins, followed by stimulation with LPS (10 μg/mL) for 24 hrs. (**A, B**) Western blot was used to detect the expression of NF-κB in BV2 cells. (**C, D, E, F, G, H and I**) The levels of TNF-α, IL-6, IL-1β, IL-2, IL-12, MCP-1 and IL-10 in the culture media were measured with ELISA. **P < 0.01 compared with control group, ##P < 0.01 compared with LPS group, ^^P<0.01 compared with LPS + Dex group.

the LPS group, compared with the control group. However, the number of phagocytic cells was significantly decreased in the presence of Dex, which was further enhanced following transfection with miR-340 mimics (Figure 6A and B). These data indicated that Dex could inhibit LPS-induced phagocytosis in BV2 cells.

Discussion

Previous study demonstrated that surgical could trigger systemic and neuroinflammation, which could induce POCD.[34] It has been shown that Dex could alleviate POCD.[35] Our results indicated that Dex reduced NF-κB level in LPS-stimulated BV2 cells. In addition, overexpression of miR-340 could enhance the anti-inflammatory effect of Dex in LPS-stimulated BV2 cells via inhibiting NF-κB (Figure 7).

In neuroinflammatory process, NF-κB plays a key role in neuroinflammation-mediated neurodegeneration.[36] Upregulation of NF-κB is closely related to the occurrence of Alzheimer's disease, and inhibition of NF-κB might be

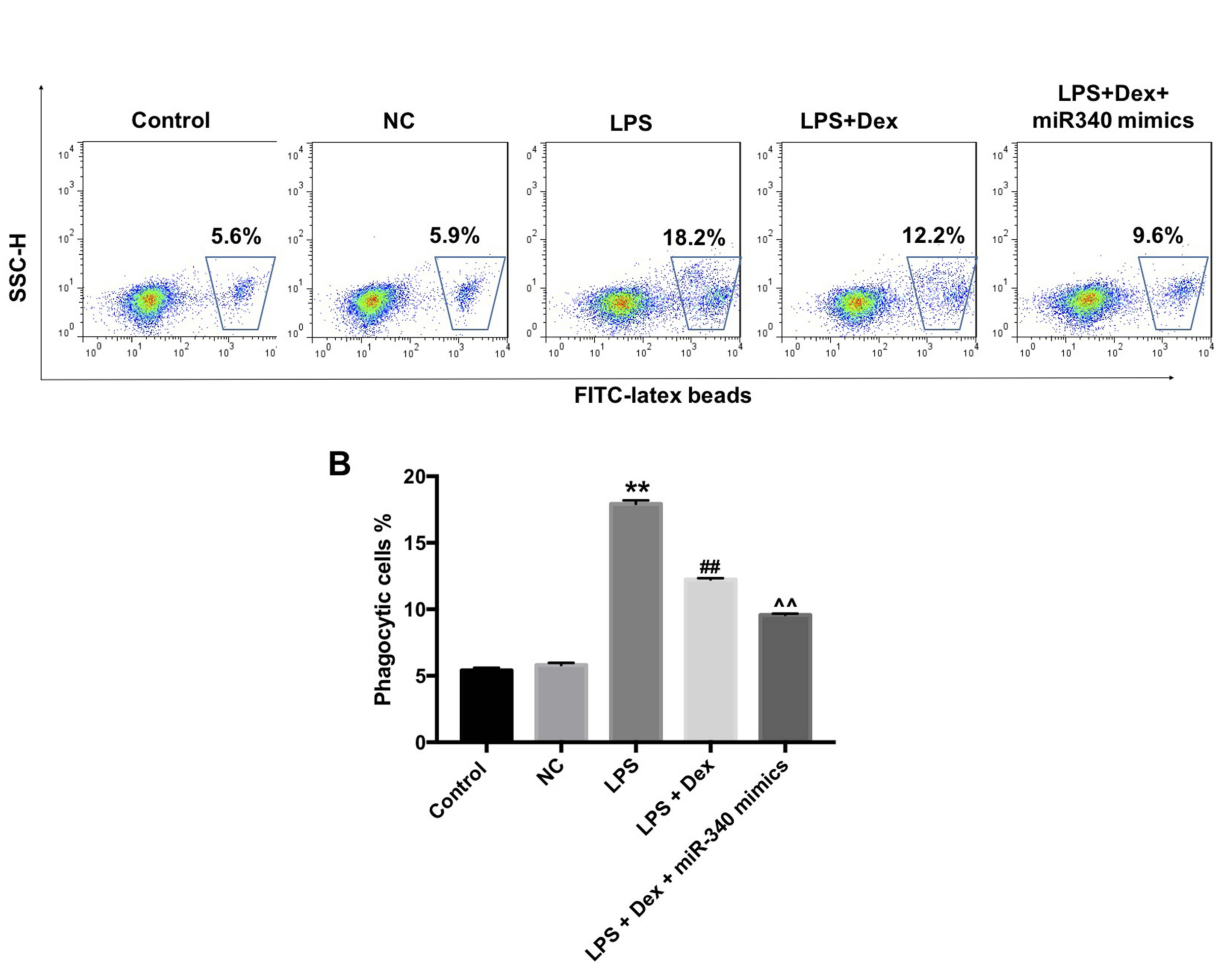

Figure 6 Dex inhibited LPS-induced phagocytosis in BV2 cells. BV2 cells were transfected with miR-340 mimics for 24 hrs and then incubated with Dex for 30 mins, followed by stimulation with LPS (10 μg/mL) for 24 hrs. (**A, B**) BV2 cells were incubated with FITC-Latex beads for 1 hr and then the uptake of beads was detected by flow cytometry. **P < 0.01 compared with control group, ##P < 0.01 compared with LPS group, ^^P<0.01 compared with LPS + Dex group.

a therapeutic method to treat Alzheimer's disease.[37] It has been indicated that proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 and MCP-1, play an important role in the NF-κB signaling pathway.[38] NF-κB could activate the proinflammatory cytokines TNF-α, IL-1β, IL-6 and MCP-1 in cells, which could induce inflammation response.[38] Previous studies indicated that the levels of proinflammatory cytokines IL-12 and IL-6 were upregulated, and the level of anti-inflammatory cytokine IL-10 was downregulated in LPS-stimulated BV2 cells.[39,40] High levels of pro-inflammatory cytokines in BV2 cells could contribute directly to neuroinflammation.[40] In the present study, Dex significantly decreased the expression of NF-κB in LPS-stimulated BV2 cells. Meanwhile, LPS significantly increased the production of TNF-α, IL-6, IL-1β, IL-2, IL-12 and MCP-1 in BV2 cells. Chen et al found that Dex reduces lung injury via inhibiting NF-κB signaling pathway, which was consistent with our results.[41] These data indicated that Dex could decrease neuroinflammation in LPS-stimulated BV2 cells via inhibiting NF-κB.

Evidence indicates that miRNAs play an important role in inflammation response, which could mediate gene expression and function.[42] Overexpression of miR-146a could alleviate inflammation response in mice with POCD, as evidenced by a decreased level of NF-κB and proinflammatory cytokines (IL-1β and IL-6).[43] However, upregulation of miR-30b could induce cognitive dysfunction in AD via increasing the level of NF-κB.[44] Previous study indicated that overexpression of miR-340 could alleviate inflammation as well as the levels of TNF-α and IL-6 in the model of chronic constriction injury.[45] Meanwhile, miR-340 could inhibit the progression of glioblastoma cells.[33] It has been reported that NF-κB was demonstrated to regulate miR-340.[32] Currently, no reports indicated the relationship of miR-340 and NF-κB in LPS-stimulated

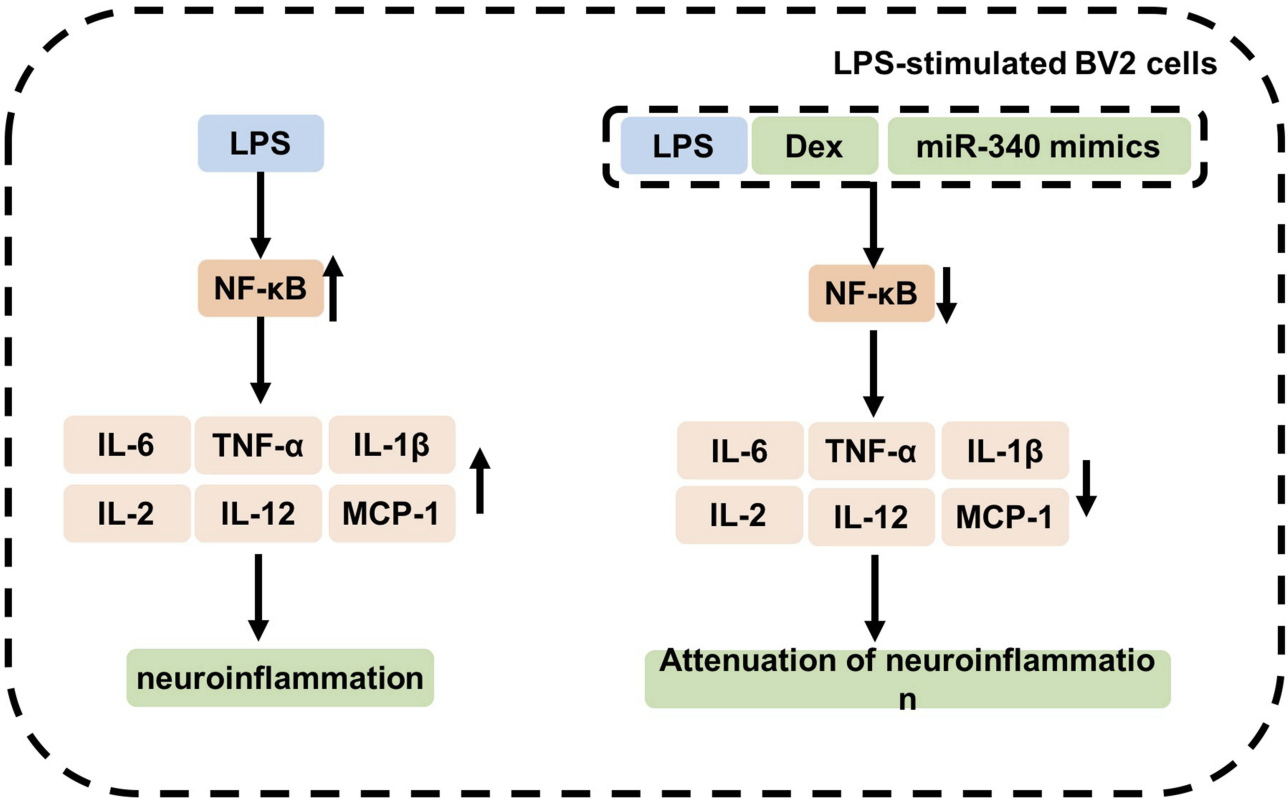

Figure 7 MiR-340 mimics exhibited anti-inflammation effect via downregulation of NF-κB pathway. The mechanism by which miR-340 mimics protected BV2 cells against LPS is shown in the figure.

BV2 cells. By analyzing the effects of miR-340 up and down-regulation in BV2 cells, we found that overexpression of miR-340 markedly decreased the level of NF-κB, according to the luciferase assay results. In this study, we found that Dex significantly increased the level of miR-340, which plays an anti-inflammatory role in BV2 cells. In addition, our results showed that the levels of NF-κB and pro-inflammatory cytokines were significantly decreased by Dex in the LPS-stimulated BV2 cells, which were further downregulated following transfection with miR-340 mimics. Our study confirmed that overexpression of miR-340 enhanced the anti-inflammatory effect of Dex in LPS-stimulated BV2 cells by decreasing the level of NF-κB and NF-κB pathway-related proinflammatory cytokines (Figure 7). The protective effect is on the basis of the inhibition of NF-κB pathway.

The limitation in our study is that no behavior tests were performed, and so we do not know whether miR-340 are associated with POCD. In addition, more inflammatory proteins, such as TLR4 and MyD88, are needed to further confirm the roles of Dex in regulating neuroinflammation in LPS-stimulated BV2 cells.

Conclusion

Our data demonstrated that Dex exerts an anti-inflammatory effect in LPS-stimulated BV2 cells. In addition, overexpression of miR-340 could enhance the anti-inflammatory effect of Dex in LPS-stimulated BV2 cells via inhibiting NF-κB pathway. Therefore, Dex may serve as a potential agent for the treatment of neuroinflammation.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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