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Suppression of central chemokine fractalkine receptor signaling alleviates amyloid-induced memory deficiency

Jiang Wu [a,b], Bihua Bie [a], Hui Yang [a], Jijun J. Xu [a], David L. Brown [a], Mohamed Naguib [a,*]

[a] Anesthesiology Institute, Cleveland Clinic, Cleveland, OH, USA
[b] College of Basic Medical Sciences, Dalian Medical University, Dalian, China

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ABSTRACT

The abnormal accumulation of amyloid fibrils in the brain is pathognomonic of Alzheimer's disease. Amyloid fibrils induce significant neuroinflammation characterized by the activation of microglia and impairment of synaptic plasticity in the brain that eventually leads to cognitive decline. Chemokine fractalkine receptor (CX3CR1) is primarily located in the microglia in the brain and its role in the amyloid fibril-induced neuroinflammation and memory deficiency remains debated. We found that bilateral microinjection of amyloid beta $(A\beta)_{1-40}$ fibrils into the hippocampal CA1 area of rats resulted in significant upregulation of CX3CR1 messenger RNA (mRNA) and protein expression (via increasing histone H3 acetylation in the *Cx3cr1* promoter region), synaptic dysfunction, and cognitive impairment, compared with the control group. Suppressing CX3CR1 signaling with CX3CR1 small interfering RNA in rats injected with $A\beta_{1-40}$ fibrils blunted $A\beta_{1-40}$-induced CX3CR1 upregulation, microglial activation, interleukin-1β expression, restored basal glutamatergic strength and electric stimuli-induced long-term potentiation, and cognitive capacities. These findings suggest that activation of CX3CR1 plays an important role in the neuroinflammatory response and Aβ-induced neuroinflammation and neurotoxicity.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by loss of memory and cognitive function. The brains of patients with AD are characterized by extensive deposits of extracellular aggregation of amyloid beta (Aβ) peptides. Misfolding of the Aβ peptides leads to the formation of amyloid fibrils, oligomers, and aggregation nuclei, which exhibit significant cytotoxicity in the brain and impair central synaptic plasticity and memory (Stefani, 2012). Accumulation of amyloid aggregates is associated with activation of microglial cells in the brain (Eikelenboom et al., 2006; Hardy and Selkoe, 2002; Wyss-Coray, 2006). There is evidence indicating that neuroinflammation plays a significant role in the pathogenesis and progression of AD (Akiyama et al., 2000; Masliah, 2008; McGeer et al., 1988; Perry et al., 2010). In brains demonstrating AD, Aβ appears to trigger microglial activation and neuroinflammation—through the production of the proinflammatory chemokines, cytokines, and neurotoxins by the activated microglia—that lead to progressive loss of neuronal circuitry, impairment of the synaptic plasticity in the brain, and eventually dementia (Barry et al., 2011; Chacon et al., 2004; Ramirez et al., 2005; Wu et al., 2013). The chemokine, fractalkine, and its receptor, CX3CR1, appear to play a central role in communication and interaction between microglia and neurons and might contribute to neuropathology of neuroinflammatory diseases such as AD (Harrison et al., 1998; Xia et al., 2000).

In the brain, the CX3CR1 expression appears to be limited to microglia whereas that of fractalkine is restricted to neurons (Cardona et al., 2006; Ransohoff, 2009). Activation of chemokine receptors results in a diverse range of intracellular signaling cascades, including phosphorylation of mitogen-activated protein kinases, formation of diacylglycerol by protein kinase C, and release of calcium from intracellular stores via phospholipase C (Old and Malcangio, 2012). Binding of fractalkine to CX3CR1-bearing microglia mediates chemotaxis of microglia to the site of injury in the brain (Ransohoff and Cardona, 2010; Re and Przedborski, 2006). It is still debated, however, whether activation of endogenous CX3CR1 signaling in microglia is neuroprotective (Cardona et al., 2006; Cho et al., 2011; Lee et al., 2010) or neurotoxic (Denes et al., 2008; Donnelly et al., 2011; Fuhrmann et al., 2010; Paolicelli et al., 2011) in the settings of neuroinflammatory and/or neurodegenerative diseases.

In mice overexpressing human amyloid precursor protein (hAPP mice), ablation of CX3CR1 decreased Aβ deposition (Lee et al., 2010) but enhanced tau pathology and worsened memory and cognitive

This work was conducted at Anesthesiology Institute, Cleveland Clinic, Cleveland, OH.

* Corresponding author at: Anesthesiology Institute, Cleveland Clinic, 9500 Euclid Avenue, NE6-306, Cleveland, OH 44195, USA. Tel.: +1 216 444 6328; fax: +1 216 636 2043.
E-mail address: naguibm@ccf.org (M. Naguib).

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functions (Cho et al., 2011). Others have shown that activation of CX3CR1 signaling appears to be neuroprotective against microglial-induced neurotoxicity (Cardona et al., 2006). In contrast, another study using 2-photon microscopy, revealed that deletion of CX3CR1 prevented neuronal loss and microglia migration in triple-transgenic mice crossed with the microglial chemokine receptor CX3CR1 knockout mouse (Fuhrmann et al., 2010). This discordancy could be attributed to the use of different models and the potential functional compensation after genetic *Cx3cr1* knockout (Gu et al., 2003; Liang and Li, 2009).

In this study, we showed that amyloid fibrils induced upregulation of CX3CR1 expression in the microglia, and we examined the effects of suppression of CX3CR1 signaling—by using small interfering RNA (siRNA)—on neuroinflammation, synaptic plasticity, and memory function in a rat model of AD.

2. Methods

2.1. Animals

All animal procedures were approved by the Animal Care and Use Committee of the Cleveland Clinic. Animals were housed the Institutional Biological Rodent Unit on a 12-hour light/dark cycle with water and food pellets available ad libitum. Adult male Sprague-Dawley rats (Charles River, Wilmington, MA, USA) weighing 200–250 g were used, and all experiments were performed during the light cycle.

2.2. Microinjection of amyloid fibrils into the hippocampal CA1 area

As described in a previous study, rats were anesthetized with sodium pentobarbital (45 mg/kg intraperitoneally) and restrained in a stereotaxic apparatus (Bie et al., 2010). $A\beta_{1-40}$ fibrils were formed as described previously (Chacon et al., 2004; Wu et al., 2013). $A\beta_{1-40}$ fibrils (10 μg/3 μL) were injected bilaterally into each hippocampus (coordinates: Bregma −3.5 mm anteroposterior, ±2.0 mm mediolateral, and −3.0 mm dorsoventral) (Paxinos and Watson, 1998; Wu et al., 2013) using a 10-μL Hamilton syringe with a 27-gauge stainless steel needle at a rate of 0.5 μL/min. This experimental paradigm has been used for modeling AD (Ahmed et al., 2010; Chacon et al., 2004; Wu et al., 2013). Artificial cerebrospinal fluid (3 μL) or reverse sequence peptide, $A\beta_{40-1}$ fibrils (10 μg/3 μL) was bilaterally injected in the same brain area in the control groups. The accuracy of the microinjection was histologically verified afterward (Bie et al., 2009a; Wu et al., 2013).

2.3. Delivery of CX3CR1 siRNA into the hippocampal CA1 area

The delivery of synthesized CX3CR1 siRNA and scrambled RNA was performed following a previously published protocol (Sioud, 2005). CX3CR1 siRNA and scrambled RNA were designed and synthesized by Santa Cruz Biotechnology, Inc (Santa Cruz, CA, USA). siRNA or scrambled RNA (5 nmol) was dissolved in the transfection medium (3 μL, Santa Cruz Biotechnology, Inc), and delivered into each side of the hippocampal CA1 area (using the same coordinates as described previously in text) every 48 hours for 4 deliveries starting from the seventh day after microinjection of amyloid fibrils in the modeled rats. The efficacy of CX3CR1 siRNA was determined by measuring CX3CR1 messenger RNA (mRNA) level and protein expression in the hippocampal CA1 area.

2.4. Morris water maze test

A cohort of rats ($n = 10$ in each group) was tested 14 days after microinjection of amyloid fibrils as described elsewhere (Wu et al., 2013). Testing was conducted in all groups at the same time of the day (9:00 AM to 12:00 noon.). The experimental apparatus consisted of a circular pool (180 cm in diameter, 45 cm high). An invisible platform (15 cm in diameter, 35 cm high) was placed 1.5 cm below the surface of the water. The swimming path of the rat was recorded by a video camera and analyzed using EthoVision XT software (Noldus Information Technology). Each rat underwent 4 trials per day for 5 consecutive days. During each trial (memory acquisition), the rats were placed into the maze consecutively from 4 random points of the pool, and were allowed to search for the platform for 120 seconds. If the rat did not find the platform within 120 seconds, they were gently guided to it. Rats were allowed to remain on the platform for 20 seconds. The latency for each trial was recorded for analysis. On Day 6, all rats were subjected to 1 probe trial (memory retrieval) in which the platform was removed, and each animal had 60 seconds to search the pool for the platform. All behavioral testing was performed by only 1 experimenter (Dr Wu), who was blinded to the different treatment groups.

2.5. Hippocampal slice preparation and whole-cell recordings

Brain slices containing the hippocampal CA1 area were prepared, as previously described, after rats completed behavioral testing (Bie et al., 2009a, 2009b, 2010, 2011b; Wu et al., 2013). The brain was quickly removed and cut on a Vibratome in cold physiological saline to obtain coronal slices (300 μm thick) containing the hippocampus. A single slice was submerged in a shallow recording chamber and perfused with warm (35 °C) physiological saline. Whole-cell voltage-clamp recordings from the CA1 area were taken using an Axopatch 200B amplifier (Molecular Devices) with 2–4 MΩ glass electrodes containing the internal solution (mM): K-gluconate or cesium methanesulfonate, 125; NaCl, 5; $MgCl_2$,1; EGTA (ethylene glycol tetraacetic acid), 0.5; Mg-ATP (ATP, Adenosine-5′-triphosphate), 2; Na_3-GTP (GTP, guanosine-5′-triphosphate), 0.1; HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 10; pH 7.3; 290–300 mOsmol. A seal resistance of ≥2 GΩ and an access resistance of 15–20 MΩ were considered acceptable. The series resistance was optimally compensated by ≥70% and constantly monitored throughout the experiments. The membrane potential was held at −70 mV, unless otherwise stated, throughout the experiment. Schaffer collateral commissural fibers were stimulated by ultrathin concentric bipolar electrodes (FHC Inc, Bowdoinham, ME, USA), and the excitatory postsynaptic currents (EPSCs) were recorded in the presence of $GABA_A$ (gamma-aminobutyric acid receptor type A) receptor antagonist bicuculline (30 μM) in the CA1 area. The evoked EPSCs were filtered at 2 kHz, digitized at 10 kHz, and acquired and analyzed using Axograph X software. If synaptic transmission was stable (<15% change in EPSC amplitude over 15 minutes), long-term potentiation (LTP) was induced by a single high-frequency electric stimuli train (100 Hz for 1 second) (Shipton et al., 2011). All electrophysiological experiments were performed at room temperature (23 ± 2 °C).

2.6. Immunohistochemistry

Immunostaining on the serial sections containing the hippocampal CA1 area in all groups (30 μm) was performed as previously described (Bie et al., 2010; Wu et al., 2013). Rats in all groups were deeply anesthetized with 60 mg/kg sodium pentobarbital and perfused transcardially with 0.1 M phosphate buffered saline (PBS) followed by 4% formalin. The brain was collected, postfixed in the same fixative for 4 hours, and then cryoprotected in 30% sucrose in PBS for 3 days. Serial sections (30 μm, 15–20 per rat) containing the hippocampal CA1 area were cut from the fixed brain. The sections were incubated for at least 1 hour in 0.01 M PBS with 0.3% Triton