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Lycopene mitigates β-amyloid induced inflammatory response and inhibits NF-κB signaling at the choroid plexus in early stages of Alzheimer's disease rats

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Abstract

The choroid plexus is able to modulate the cognitive function, through changes in the neuroinflammatory response and in brain immune surveillance. However, whether lycopene is involved in inflammatory responses at the choroid plexus in the early stages of Alzheimer's disease, and its molecular underpinnings are elusive. In this rat study, lycopene was used to investigate its protective effects on inflammation caused by β-amyloid. We characterized the learning and memory abilities, cytokine profiles of circulating TNF-α, IL-1β and IL-6β in the serum and the expressions of Toll like receptor 4 and nuclear factor-κB p65 mRNA and protein at the choroid plexus. The results showed that functional deficits of learning and memory in lycopene treatment groups were significantly improved compared to the control group without lycopene treatment in water maze test. The levels of serum TNF-α, IL-1β and IL-6β were significantly increased, and the expressions of TLR4 and NF-κB p65 mRNA and protein at the choroid plexus were up-regulated, indicating inflammation response was initiated following administration of $A\beta_{1-42}$. After intragastric pretreatment with lycopene, inflammatory cytokines were significantly reduced and lycopene also reversed the $A\beta_{1-42}$ induced up-regulation of TLR4 and NF-κB p65 mRNA and protein expressions at the choroid plexus. These results provided a novel evidence that lycopene significantly improved cognitive deficits and were accompanied by the attenuation of inflammatory injury *via* blocking the activation of NF-κB p65 and TLR4 expressions and production of cytokines, thereby endorsing its usefulness for diminishing β-amyloid deposition in the hippocampus tissues.

Keywords: Choroid plexus; Lycopene; NF-κB; Inflammation; $A\beta_{1-42}$; Neuroprotection

1. Introduction

Alzheimer's disease (AD), the most common type of dementia, is a degenerative disease of the central nervous system characterized by progressive cognitive and behavioral impairments [1,2]. Regardless of the attention given to increased amyloid β protein (Aβ) formation and toxicity in the brain, neuroinflammation is recognized as an early and essential mediator of brain pathology and behavior alterations in AD [3,4]. Noticeably, increased amyloid load is accompanied by marked inflammatory alterations, both at the level of the brain parenchyma and at the barriers of the brain [5,6]. Recent studies have highlighted the alterations observed at the choroid plexus (CP), which forms the blood-cerebrospinal barrier (BCSFB), and their impact on brain functions in aging and in AD [7–9]. Additionally, the CP is an important structure as an inflammatory sensor that detects signals originating from both the peripheral and the central nervous system [10–13]. Therefore, understanding the responses at the CP may unravel novel pathways of relevance for the pathophysiology of AD. Interestingly, the role of the CP in the neuroinflammatory responses in AD, as well as its connection with impaired memory and cognition, is still poorly understood.

Lycopene (LYCO), an aliphatic hydrocarbon carotenoid, present in the ripened tomatoes as a potential useful agent in the management of neurodegenerative disorders because of its strong antioxidant property in several AD models [14–16]. Although lycopene in AD has been extensively investigated so far and well established, its barrier protective effects and underlying mechanisms are not fully identified. Recently, a significant result demonstrates that Toll like receptor-4 (TLR4) and NF-κB-dependent inflammatory response at the CP is associated with regulation of the internal brain milieu and cerebrospinal fluid (CSF) secretion [17]. The CP secretes higher volumes of CSF than any other epithelium and simultaneously functions as the blood-CSF barrier to gate immune cell entry into the central nervous system. Therefore, TLR4 represents a suitable target for therapeutic intervention within the disease progression and targeting them carefully could increase Aβ autophagy and phagocytosis or reduce inflammatory responses. However, whether lycopene is involved in suppressing Aβ uptake or inflammatory responses in the early stages of AD, and its molecular underpinnings at the CP are elusive. The present study was carried out to elucidate the neuroprotective effect of lycopene against

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the Aβ-induced cognitive impairment, the activation of TLR4 and NF-κB at the CP in the early stages of AD. These considerations have significant implications on the development of TLR4-based novel therapies aimed at implementing a controlled manipulation of this receptor at the CP in relation to the disease stage.

2. Materials and methods

2.1. Animals

Thirty two adult male Wistar rats (SPF class, weighing 250–300 g) were provided by the Chinese Academy of Sciences. The animal experiments were conducted to follow the guidelines established by the Chinese Committee on Experimental Animal Supervision. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. All rats were randomly divided into four groups (control, $A\beta_{1-42}$, lycopene + $A\beta_{1-42}$ and lycopene group) according to body weight. The rats in lycopene + $A\beta_{1-42}$ and lycopene groups were treated by intragastric of lycopene (5 mg/kg body weight) daily for 21 days before $A\beta_{1-42}$ injection, while the rats in control and $A\beta_{1-42}$ groups were treated by intragastric of 0.5% CMC-Na (sodium carboxyl methyl cellulose) [16].

2.2. Preparation of Aβ, lycopene and surgery

$A\beta_{1-42}$ and lycopene were purchased from Sigma Chemicals Co., St. Louis, Mo, USA. $A\beta_{1-42}$ (3 nmol/3 mL) was prepared in artificial cerebrospinal fluid (ACSF, in mmol/L: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 dextrose) and was administered intracerebroventricular injection (ICV) at a dose of 3 μL using Hamilton syringe. Lycopene was dissolved in 0.5% CMC-Na and administered at a dose of 5 mg/kg body weight for 21 days. All animals were anesthetized with thiopental sodium (45 mg/kg body weight) and positioned in a stereotaxic apparatus. The skulls of rats were opened and drilled with one hole (anteroposterior 1.2 mm from Bregma, mediolateral 2.0 mm, dorsoventral 4.0 mm) using a stereotaxic frame (Narishige, Tokyo, Japan). A miniosmotic pump (Durect, Cupertino, CA, USA) containing either $A\beta_{1-42}$ solution or vehicle alone was quickly implanted into the neck of the rats. The outlet of the pump was inserted 3.5 mm into the lateral cerebral ventricle and fixed at screws with dental cement. $A\beta_{1-42}$ solution was spontaneously infused to lateral cerebral ventricle by miniosmotic pump for 21 days. Special care of the animals was taken during the post-operative period.

2.3. Assessment of behavioral parameters

Twenty-one days later, the learning and memory abilities of rats were evaluated by the Morris water maze [18]. Briefly, these animals were released from four randomly assigned start positions respectively. Each rat was trained for four consecutive days to find the hidden platform. Each trial was concluded when the platform was reached within the time limit of 120 s. If the rats failed to reach the platform within this time period, they were guided to the platform and allowed to stay in it for 30 s. The four random consecutive trials of each day were video-captured with a video-tracking system. The full acquisition time course was recorded. The final readout included the escape latency, the distance to arrive the hidden platform, and the frequency of the rat spanning the place where the platform laid.

2.4. Measurement of cytokines in serum by ELISA

Biochemical tests were conducted 24 h after the last behavioral test. The blood was collected from arteria cruralis of rats. Briefly, injector was used to take blood after the skin of the rat was cut and the arteria cruralis was exposed by blunt dissection. Blood was centrifuged at 1, 250×*g* for 10 min at 4°C. Serum levels of circulating TNF-α, IL-1β and IL-6β were measured in duplicate by immunoassay using a commercially available enzyme-like immunosorbent assay (ELISA) kit (Groundwork Biotechnology Diagnosticate, USA). The procedure was strictly according to the manufacturers' instructions. The resulting color reaction was measured at 450 nm with an ELISA reader (Infinite M200, TECAN, Switzerland). Values were expressed as ng/L serum.

2.5. Tissue sample collection and storage

Under deep anesthesia, rats were transcardially perfused with 0.9% saline and the brains were removed from the skull. For protein levels analysis and detection of gene expression by real-time quantitative PCR (RT-qPCR), the CP samples from each brain ventricle of the same mouse were rapidly removed under a conventional light stereomicroscope (SZX7, Olympus, Hamburg, Germany), pooled, snap-frozen and stored at −80°C. Specific brain areas, namely the right hippocampus (R-HPC) were obtained by macrodissection, snap-frozen and stored at −80°C for RNA extraction and gene expression analysis.

2.6. Detection of gene expression by real-time quantitative PCR (RT-qPCR)

Total RNA was purified by using Trizol (Invitrogen, Carlsbad, CA, USA). Real-time PCR of cDNA was performed (ABI PRISM 7500 Sequence Detection System, Applied Biosystems, Grand Island, NY, USA) using forward and reverse primer sequences. Data were analyzed using a comparative critical threshold (Ct) method where the amount of target normalized to the amount of endogenous control (β-actin) and relative to the control sample was determined by the $2^{-\Delta\Delta Ct}$ method. The mRNA expressions of TLR4, p65 subunit of NF-κB, and β-actin in CP were measured. The forward and reverse primer sequences were as follows (5′ to 3′):

TLR4: TGGGTCAAGGAACAGAAGCA and TCACACTGACCACTGACACA, p65: CACCGGATTGAAGAGAAGCG and AAGTTGATGGTGCTGAGGGA β-actin: CACGATGGAGGGGCCGGACTCATC and TAAAGACCTCTATGCCAACACAGT. The annealing temperature for TLR4, p65 subunit of NF-κB, and β-actin was 53°C, 57°C and 58°C, respectively. After 35 cycles, amplification products were electrophoresed on a 2.0% agarose gel. Then FluorChem FC2 software (AlphaInnotech, America) was used to photo and analyze results.

2.7. Western blot analysis

The CP and brain tissues were harvested and washed with phosphate buffered saline, and homogenized in lysis RIPA buffer containing 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris–HCl (pH 7.4), 1 mM DTT, and protease inhibitors. The homogenate was kept at 4°C for 40 min, and then centrifuged at 8000×*g* for 15 min. Supernatant was separated and collected for protein analysis. Nucleoprotein was extracted by kit (Novagen, Germany). The protein concentration was determined by using the BCA (bicinchoninic acid) protein assay kit (Pierce Biotechnology, USA). 50 μg of protein was loaded and separated by a 10% or 12% SDS-acrylamide gel electrophoresis and transferred to polyvinylidene fluoride blots at the voltage of 60 V for 2 h. The transferred membrane was blocked by fresh blocking buffer (Tris-buffered saline, containing 5% nonfat dry milk) at room temperature for 1 h. The different primary antibodies for anti-TLR4 (1:1000, Proteintech, USA), anti-NF-κB-p65 (1:1000, Abcam), anti-β-APP (1:1000, Abcam), anti-PS-1 (1:2000, Abcam) or anti-β-actin (1:2000, Abcam) were added to the membrane and incubated for 12 h at 4°C. After completing the primary antibody reaction and washing with appropriate buffer, the secondary antibodies were added and incubated for 1 h (1:5000 in a 1% non-fat milk solution). The membrane was finally washed with TBST. ProtoBlot®II kit (Promega, USA) was used to detect and FluoChem®FC2 (Alpha Innotech) was used to photo and analyze results.

2.8. Statistical analysis

Data were analyzed with the software SPSS 11.5 (SPSS, Chicago, IL, USA). Normal distribution of data was first tested by Shapiro–Wilk test, $P>.05$ was accepted. Data were expressed as mean ± SE. Statistical analysis was performed using analysis of variance (ANOVA), followed by post hoc Duncan's multiple range test. *P* value of less than .05 was considered statistically significant.

3. Results

3.1. Spatial navigation task in ICV $A\beta_{1-42}$ treated rats

Rats movement track was shown in Fig. 1a. In the fifth day, compared to the control group, the $A\beta_{1-42}$ group rats showed significantly prolonged escape latency ($[F_{(3,\ 28)}=4.96,\ P=.038<.05]$). However, the escape latency of rats in the LYCO + $A\beta_{1-42}$ and LYCO groups were much shorter than those of the $A\beta_{1-42}$ group ($[F_{(3,\ 28)}=5.12,\ P=.032<.05]$; $[F_{(3,\ 28)}=5.25,\ P=.030<.05]$, respectively) (Fig. 1b and c). Along with these results, the total distance to arrive the hidden platform in $A\beta_{1-42}$ group rats showed significantly increased when compared to the control group ($[F_{(3,\ 28)}=5.74,\ P=.026<.05]$). However, compared to the $A\beta_{1-42}$ group, the total distance to arrive the hidden platform in the LYCO + $A\beta_{1-42}$ and LYCO groups showed significantly decreased ($[F_{(3,\ 28)}=5.66,\ P=.027<.05]$; $[F_{(3,\ 28)}=5.82,\ P=.022<.05]$, respectively) (Fig. 1d).

3.2. Cytokine production

Compared to the control group, the levels of serum TNF-α, IL-1β and IL-6β showed significantly increased in $A\beta_{1-42}$ group rats ($[F_{(3,\ 28)}=4.78,\ P=.042<.05]$; $[F_{(3,\ 28)}=4.92,\ P=.040<.05]$; $[F_{(3,\ 28)}=4.89,\ P=.041<.05]$, respectively) (Fig. 2). However, compared to the $A\beta_{1-42}$ group rats, the levels of serum TNF-α, IL-1β and IL-6β showed significantly decreased in $A\beta_{1-42}$ + LYCO group rats ($[F_{(3,\ 28)}=5.34,\ P=.029<.05]$; $[F_{(3,\ 28)}=6.20,\ P=.018<.05]$; $[F_{(3,\ 28)}=3.85,\ P=.047<.05]$, respectively) (Fig. 2). Also, compared to the $A\beta_{1-42}$ group rats, the levels of serum TNF-α, IL-1β and IL-6β showed significantly