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Progesterone suppresses $A\beta_{42}$-induced neuroinflammation by enhancing autophagy in astrocytes

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ABSTRACT

Autophagy is an intracellular catabolic mechanism essential for recycling intracellular unfolding protein and eliminating toxic protein aggregates. Several studies have shown that deficient autophagy is implicated in the development of Alzheimer's disease (AD) progression. To date, rapidly emerging evidence suggests that neurosteroid progesterone (PG) may play an important role in ameliorating AD. However, the role of PG and its neuroprotective mechanism in regulating autophagy still require further investigation. Here, we investigated the protective effects of PG against Aβ-induced inflammatory responses in astrocytes and its underlying mechanism in mediating autophagy. Remarkably, Aβ induced astrocyte dysfunction in autophagic activation and up-regulated inflammatory secretion. However, the autophagy inducer rapamycin (RAPA) significantly suppressed Aβ-induced inflammation in astrocytes. In astrocytes, treatment with Aβ caused autophagy deficiency, whereas PG significantly increased autophagy activation. Finally, PG suppressed Aβ-induced neuroinflammatory production via enhancing autophagy together with regulating mTOR signaling. Taken together, these results show that autophagy is a vital mechanism against Aβ-induced neuroinflammatory responses in astrocytes and demonstrate the potential neuroprotective mechanism of PG in suppressing neuroinflammatory responses by enhancing autophagy. Therefore, uncovering the neuroprotective mechanism of PG may provide new insight into novel therapies for the amelioration of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder with gradual decline in cognitive processes and is pathologically characterized by intracellular neurofibrillary tangles, deposition of β-amyloid (Aβ) and excessive neuroinflammation [1]. Remarkably, neuroinflammation is a prominent feature in AD progression [2]. While inflammatory responses are generally beneficial for self-protection, this process needs to be tightly regulated to avoid a state of sustained inflammation, which is potentially self-damaging. Moreover, accumulating evidence indicates Aβ-induced glial-mediated neuroinflammatory responses highly contribute to cognitive decline and neurodegeneration [3]. The mechanisms of AD have been systematically studied, and several molecular pathways have been discovered, but the definitive mechanisms underlying glial-mediated inflammatory responses remain incompletely understood to date.

Astrocytes are the most abundant glial cell in the central nervous system and dynamically endow neurons with trophic support and modulate information processing [4]. However, the prolonged and widespread activation of astrocytes is observed in AD brain, in which the severity of glial activation correlates with cognitive decline [5]. In this state, reactive astrocytes, as opposed to quiescent astrocytes, may produce more proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ [6]. Moreover, proinflammatory molecules released by reactive astrocytes can amplify proinflammatory responses by further activating and recruiting other glial cells and elevate cytotoxic factors Aβ production in neurons as well [7]. Therefore, an increasing number of studies have focused on the above findings to examine intracellular pathological changes or determine whether reactive astrocytes themselves are not able to resist Aβ-induced neurotoxicity.

Autophagy is an evolutionarily conserved lysosome-dependent system that regulates protein homeostasis in the removal of intracellular unfolding protein and damaged organelles. During autophagy, target proteins or organelles are selectively distinguished and transfer specific cargo into double-membrane autophagosomes for lysosomal degradation. This selective removal of autophagic substrates is regulated by P62/SQSMT1 (P62), which is associated with the

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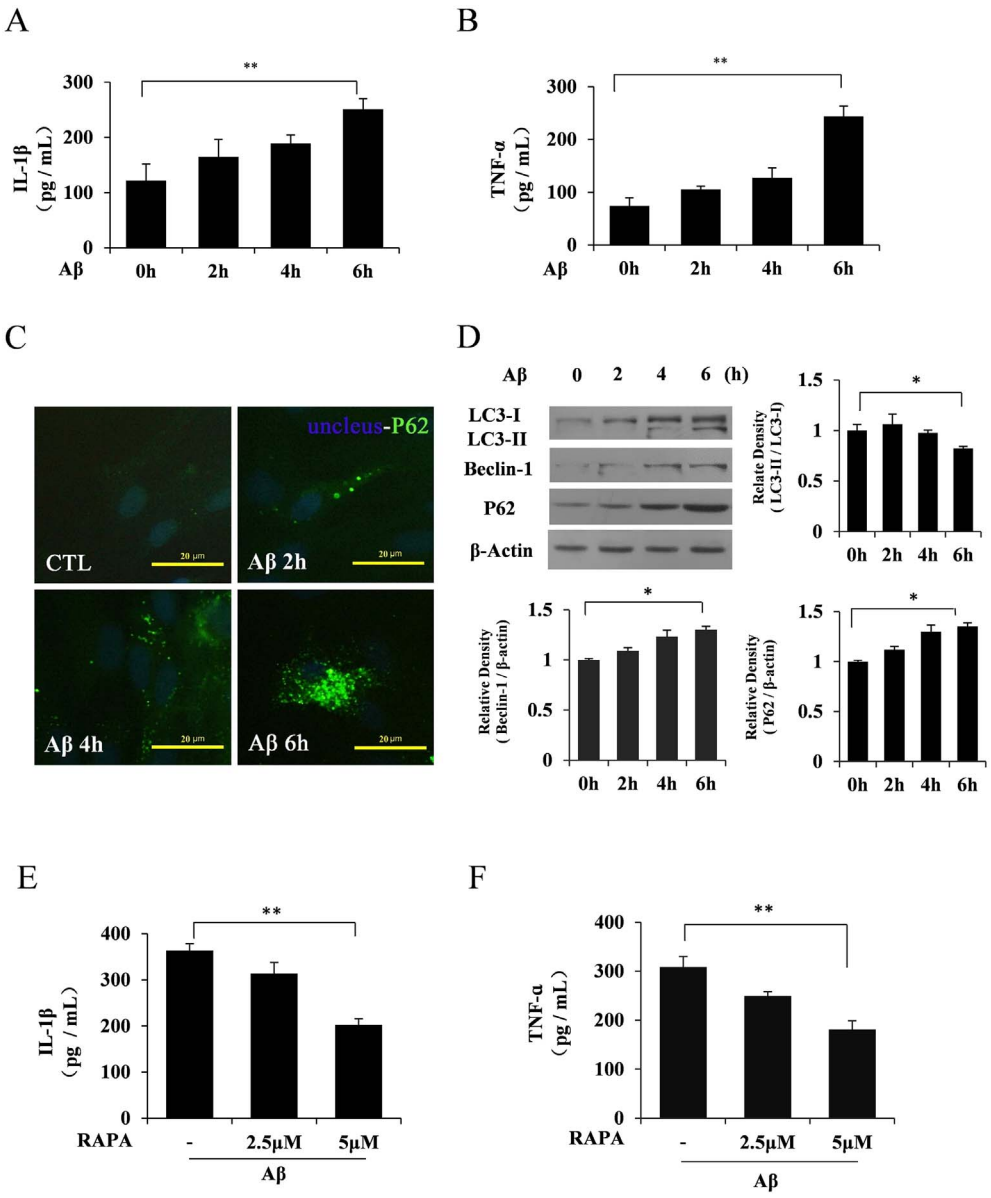

Fig. 1. Aβ-induced inflammatory cytokines release partially subjects to the dysfunction of autophagy in astrocytes.
A, B. Aβ exacerbates IL-1β and TNF-α release in astrocytes. ELISA assay was used to detect the level of IL-1β (A) and TNF-α (B) in the medium of treated astrocytes following Aβ treatment for indicated time (**$p < 0.01$, compared with non-treated group, statistical analysis was performed by Student's *t*-test). C. Aβ causes abnormal autophagic protein P62 aggregation in astrocytes. Astrocytes were treated with Aβ for indicated time, and then the cells were immunostained against antibodies P62 (green), DAIP was used to stain nuclei (blue). D. Aβ induced autophagic dysfunction in astrocytes. Western blotting was used to detect LC3, Beclin-1 and P62 expression in astrocytes (*$p < 0.05$, compared with non-treated group, statistical analysis was performed by Student's t-test). E, F. Activation of autophagy partially suppresses Aβ-induced inflammation in astrocytes. ELISA assay was used to detect the level of IL-1β (E) and TNF-α (F) in the medium of treated astrocytes (**$p < 0.01$, compared with Aβ-treated group, statistical analysis was performed by one-way ANOVA). Data represent the means ± SD of three independent determinations (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

autophagic marker microtubule associated protein light chain 3 (LC3) [8]. Previous studies have shown that deficient autophagy results in the pathogenesis of neurodegenerative diseases, especially at the late stages of AD [9,10]. Indeed, there is significant support for the possibility of defective autophagy in AD. Transgenic APP/PS1 mice show the impaired acidification of lysosomes, which is necessary for the autophagy system. The impaired proteolysis of substrates may compromise autophagy, resulting in the prevalent accumulation of autophagic vesicles (AVs) in neurites surrounding amyloid plaques [11]. This finding demonstrates that AD-associated impairment in autophagy occurs due to the excessive deposition of improperly modified proteins, which appears to trigger potential cytotoxicity. Additionally, autophagy inducer rapamycin (RAPA) recovers the learning and memory process in the course of the inhibition of Aβ and tau cytotoxicity by interfering with several signals, including neuroinflammation [12]. This finding indicates that autophagy is likely to degrade the damaged proteins that potentially induce AD-associated proinflammatory responses. We hypothesized that there is a close relationship between autophagy deficiency and Aβ-induced cytotoxicity, particularly glial-mediated inflammatory responses. Therefore, therapeutic strategies to regulate autophagy might be helpful in seeking effective new target treatments for AD.

Recently, the neurosteroid progesterone (PG) has been reported in numerous studies for its neuroprotective characteristics against several neurodegenerative disorders [13–15]. Impressively, PG exhibits a unique therapeutic function in ameliorating AD [16–18], but the potential neuroprotective mechanism has not been demonstrated. Our previous findings have indicated that PG exhibits a unique therapeutic function against Aβ-induced neuroinflammation; however, the sequence of events leading to neuroinflammation remains unclear. Particularly, the role of PG and its neuroprotective mechanism in regulating autophagy still need further investigation. In consideration of this issue, we treated primary astrocytes with PG, investigated whether PG exhibited neuroprotection to activate autophagy, and discussed the potential molecular mechanism in suppressing Aβ-induced neuroinflammatory responses in astrocytes. Hence, we speculated that neurosteroid PG may represent a new potential protective mechanism in suppressing Aβ-induced astrocytic neuroinflammation.