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Corticotropin-releasing factor receptor-1 antagonism mitigates beta amyloid pathology and cognitive and synaptic deficits in a mouse model of Alzheimer's disease

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Abstract

Introduction: Stress and corticotropin-releasing factor (CRF) have been implicated as mechanistically involved in Alzheimer's disease (AD), but agents that impact CRF signaling have not been carefully tested for therapeutic efficacy or long-term safety in animal models.

Methods: To test whether antagonism of the type-1 corticotropin-releasing factor receptor (CRFR1) could be used as a disease-modifying treatment for AD, we used a preclinical prevention paradigm and treated 30-day-old AD transgenic mice with the small-molecule, CRFR1-selective antagonist, R121919, for 5 months, and examined AD pathologic and behavioral end points.

Results: R121919 significantly prevented the onset of cognitive impairment in female mice and reduced cellular and synaptic deficits and beta amyloid and C-terminal fragment-β levels in both genders. We observed no tolerability or toxicity issues in mice treated with R121919.

Discussion: CRFR1 antagonism presents a viable disease-modifying therapy for AD, recommending its advancement to early-phase human safety trials.

Keywords: Alzheimer's disease; R121919; Corticotropin-releasing factor receptor; Corticotropin-releasing hormone; Hippocampus; Cognitive deficits; Synaptic deficits; Stress; Beta amyloid

1. Background

The neurodegenerative process in Alzheimer's disease (AD) is characterized by progressive accumulation of beta amyloid (Aβ) protein and hyperphosphorylated forms of tau protein, leading to synaptic dysfunction and cognitive impairment. Recent work has implicated environmental factors, prominently including stress, as conferring susceptibility to AD pathogenesis [1]. In addition to data demonstrating that AD mouse models have perturbations in central stress signaling and display increased anxiety behavior [2–4], epidemiologic work demonstrates that individuals prone to experience psychological distress or anxiety are more likely to be diagnosed with AD than age-matched controls [5,6] and exhibit more rapid rates of cognitive decline [6].

Corticotropin-releasing factor (CRF) is best known as the hypothalamic neuropeptide initiates the endocrine stress response via the type 1 corticotropin-releasing factor

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receptor (CRFR1), a G protein–coupled receptor (GPCR) positively coupled to adenylate cyclase [7]. CRFR1 is also expressed widely in the brain, including AD-relevant regions as isocortex, hippocampus, and amygdala [8]. A substantial number of studies demonstrate a role for CRF and CRFR1 signaling on AD end points [4,9–13].

To assess the efficacy of CRFR1 antagonism on cognitive and pathologic end points, we used a double transgenic AD mouse model (PSAPP) that develops Aβ pathology in the cortex and hippocampus beginning at 3–4 months of age in both genders and cognitive impairment in females by 6 months of age [14,15]. We took advantage of data from recent clinical trials suggesting that anti-Aβ treatments may be effective in humans when administered at preclinical/predementia stages of AD (rather than after cognitive symptoms are present [16]) and used a preclinical prevention paradigm similar to that of current anti-Aβ AD prevention trials [17] to administer a second generation, small-molecule CRFR1 antagonist to groups of 30-day-old AD mice daily for 5 months. Using this strategy, we find that CRFR1 antagonism is a safe and viable disease-modifying treatment for AD.

2. Methods

2.1. PSAPP mice

An AD-Tg mouse model (B6.C3-Tg [APPswe, PSEN1dE9] 85Dbo/Mmjax, stock no. 004462) and WT mice (C57BL/6J, stock no. 000664) were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and bred in-house. Male and female PSAPP mice, which contain a chimeric mouse/human *APP* gene co-expressed with a mutant human *PS1* gene, were used [14]. WT littermates were used as control. All mice were weaned at 21 days of age and entered the study at 30 days of age. Mice were housed (2–4 mice/cage) in a temperature-controlled room (22°C) with a 12-h light-dark cycle. A total of 102 mice, with individual group sizes per condition ranging from 11 to 15 mice were randomly assigned to either drug or vehicle arms based on gender and transgenic status. The UCSD IACUC approved all experimental protocols.

2.2. R121919 administration

For pharmacologic blockade of CRFR1, we used the well-characterized, small-molecule CRFR1-selective antagonist, R121919 [18]. R121919 was dissolved in a vehicle solution composed of 0.3% tartaric acid and 5% vol/vol polyethoxylated castor oil. Vehicle solution was used as a control and administered as prepared previously without R121919. Both R121919 and vehicle solution were mixed by vortexer and sonicator to ensure a complete mixing. The final pH of the vehicle or R121919 was at pH 3. Mice were given subcutaneous injections of vehicle or R121919 (20 mg/kg/d) for 150 days. The 20 mg/kg/d was chosen based on the efficacy of this dose to antagonize a variety of stress-related end points [12,13].

2.3. Morris water maze

The morris water maze (MWM) was used to test spatial learning and memory as a function of R121919 treatment. After basic training in the paradigm (visible platform), a probe test and spatial learning tasks were performed. Mice were given four 90-second trials per day for eight consecutive days. In the second spatial learning test, the platform was relocated into a new quadrant each day. For this task, mice were given four trials per day (90 seconds per trial) to search for the relocated platform and each mouse was released into the pool after 10 seconds of inter-trial interval (ITI) at the same start location. Testing involved placing each mouse in the tank at water-level, facing the pool wall, and at one of two start positions equidistant from the platform. Video tracking was initiated once the mouse was released and terminated automatically when the animal remained on the platform for >3 seconds. Mice were allowed to remain on the platform for a total of 10 seconds during the ITI.

2.4. Sample collection

After behavioral testing, mice were sacrificed under deep anesthesia with isoflurane, trunk blood was collected, and plasma and serum were frozen and stored at −80°C. Brains were rapidly removed after decapitation, and the right hemisphere cortex and hippocampus were harvested on ice for biochemical assays [12,13], whereas the left hemisphere was saved for immunohistochemical analyses. Livers were snap frozen and stored at −20°C for pathologic analyses.

2.5. Immunohistochemical analyses

For detection of diffuse and neuritic Aβ plaques, an N-terminal–specific anti-human Aβ monoclonal antibody (82E1) [19] and stereological methods [20] were used. To assess changes in cell and synaptic densities in the cortex and hippocampus, MAP2 and anti-synaptophysin antibodies were used. Details of immunohistochemical procedures, quantification, and stereological analyses are provided in supplemental methods.

2.6. Western blot

To analyze changes in both full-length amyloid precursor protein (APP) and C-terminal fragments (CTFs) of APP, 22C11 and CT-15 antibodies were used, respectively. Aβ peptides were detected with 82E1. Details of Western blot procedures and quantification are described in supplemental methods.