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Research Report

Genistein inhibits aggregation of exogenous amyloid-beta$_{1–40}$ and alleviates astrogliosis in the hippocampus of rats

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ABSTRACT

We addressed the question of whether injection of Amyloid beta (Aβ)$_{1–40}$ in the rat brain is associated with pathology in the hippocampus, and if genistein has any protective effect against the neuronal damage caused by Aβ$_{1–40}$. Genistein is a plant-derived compound with a structure similar to that of the female sex hormone estrogen and it was recently shown that pretreatment with a single dose of genistein ameliorated learning and memory deficits in an (Aβ)$_{1–40}$ rat model of Alzheimer's disease. Here, we report that injection of the amyloid peptide into the hippocampus of rats led to formation of Aβ$_{1–40}$ positive aggregates close to the lateral blade of the dentate gyrus (DGlb). We also observed the following in the hippocampus: extensive cell death in the DGlb ($P<0.0001$), CA1 ($P=0.03$), and CA3 ($P=0.002$); an increased number of iNOS-expressing cells ($P=0.01$) and gliosis. Genistein given to rats by gavage 1 h before injection of Aβ$_{1–40}$ inhibited the formation of Aβ$_{1–40}$ positive aggregates in the brain tissue and led to increased number of nNOS$^+$ ($P=0.0001$) cells in the hippocampus compared to sham-operated genistein-treated controls. Treatment with genistein also alleviated the extensive astrogliosis that occurred in Aβ$_{1–40}$-injected hippocampus to a level similar to that observed in sham-operated rats. We conclude that the neurons in the DGlb are most sensitive to Aβ$_{1–40}$, and a single dose of genistein can ameliorate Aβ$_{1–40}$ induced pathology.

1. Introduction

In patients with Alzheimer's disease (AD), the brain shows extracellular β-amyloid (Aβ) deposition as well as intracellular neurofibrillary tangles. Dystrophic neuritis, synaptic loss, and neuronal death are additional pathological hallmarks of AD. Much of the research on the pathogenesis of this disease has focused on the role of abnormally high amyloid secretion,

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Abbreviations: AD, Alzheimer's disease; iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; CA1, cornu ammonis 1; CA2, cornu ammonis 2; CA3, cornu ammonis 3; Aβ, amyloid beta; DGlb, lateral blade of dentate gyrus; DGmb, medial blade of dentate gyrus; GFAP, glial fibrillary acidic protein; CC, corpus callosum; MAP, mitogen-activated protein; NFκB, nuclear factor kappa B; MnSOD, manganese superoxide dismutase; APP, amyloid precursor protein; CrEL, Cremophor EL

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which is believed to be the central event in neuronal degeneration. In vitro, the presence of Aβ is associated with degeneration of neurites (Horiuchi et al., 2010), and overexpression of Aβ in transgenic mice induces neuronal degeneration consistent with that observed in Alzheimer's patients (Games et al., 1995). Also injection of Aβ into the rat brain causes neuronal damage (Miguel-Hidalgo et al., 1998, 2002). Even though much progress has been made in elucidating the biological mechanism of development of this disease, there is still no cure. The increasing number of patients suffering from AD throughout the world (Fratiglioni et al., 2010) indicates an urgent need for preventive measures and effective therapy.

Soy protein contains a large amount of isoflavones which are associated with a wide variety of beneficial health effects. One of the best-known isoflavones i.e. genistein is absorbed in the small intestine (Picherit et al., 2000), can be detected in plasma and serum after oral administration (Rowland et al., 2003), and crosses the blood–brain barrier (Tsai, 2005). Genistein has a structure similar to 17β-oestradiol, and it can bind to the estrogen receptors (Lephart et al., 2004). Estrogen offers some protection against Aβ-induced cell death, but it also has serious oncogenic effects on non-neuronal cells (Bang et al., 2004), and thus renders this hormone of limited use for treatment purposes. Genistein may have a beneficial influence similar to that of estrogen but without the negative side effects (Bang et al., 2004). Genistein acts via estrogen receptors to stimulate MAP kinases; these proteins activate the NFκB signaling pathway and thereby induce overexpression of manganese superoxide dismutase (MnSOD), which serves as an antioxidant in the cell (Akiyama et al., 1987; Borras et al., 2006). Recently, Huang and Zhang (Huang and Zhang, 2010) observed that chronic ingestion of genistein reduced neuronal apoptosis in the brain of ovariectomized rats. Furthermore, Valles et al. (2008) found that pretreatment with genistein attenuated Aβ-induced death of cortical neurons in vitro by lowering oxidative stress. It is plausible that genistein provides a protective effect via its anti-inflammatory influence, or via inhibition of the endoplasmic reticulum stress that arises due to accumulation of unfolded proteins (Park et al., 2010). Overall, it appears that genistein has a positive impact on various cellular mechanisms that are assumed to underlie the development of AD. Therefore, genistein may be a good candidate in the search for compounds that can be used to prevent or treat AD in the future. We have previously observed that genistein ameliorated impairment of short-term spatial memory induced by intrahippocampal injection of $A\beta_{1-40}$ in rats (Bagheri et al., 2011). In the current study, we addressed the question of whether injection of $A\beta_{1-40}$ in the rat brain is associated with pathology in the hippocampus, and if genistein has any protective effect against the neuronal damage caused by $A\beta_{1-40}$.

2. Results

The cerebrum was mechanically damaged at the site of the needle insertion, and the surrounding tissue contained many small glia-like cells. Congo-red-stained brain sections showed no apple-green birefringence in a polarizing microscope, which suggests the lack of amyloid fibrils in the tissue. The slides that were incubated without primary antibodies and served as negative controls lacked any sign of immunoreactivity. These observations were made in sections from rats in all groups and are not further mentioned below.

2.1. *Sham-operated rats*

In hippocampal sections from these rats, cresyl-violet staining indicated normal morphology (Fig. 1A1). The mean numbers (±SEM) of cells were 149±6 in CA1, 107±5 in CA3, and 306±22 in DGlb (Figs. 2A–C), and no Aβ immunoreactivity was observed in the tissue (Fig. 1A2). The $iNOS^+$ (Fig. 3A1) and $nNOS^+$ (Fig. 3A2) cells were most common in CA1, CA3, and DG, and were rare in CA2 and stratum radiatum; cells in stratum oriens showed no immunoreactivity. For the mean number of $iNOS^+$ and $nNOS^+$ cells in the hippocampus see Figs. 4A–B.

$GFAP^+$ astrocytes were observed throughout the cerebral cortex and hippocampus. Immunoreactivity was most intense in corpus callosum (CC) and the hippocampal strata cingulate and oriens, and DG polymorphic layer; less extensive in strata radiatum and molecular; and weakest in stratum lacunosum. In the cortex, immunoreactivity was greater in layers 5–6 than in superficial layers and was not detectable in stratum granular (Fig. 1A3). The reactive astrocytes exhibited stellate shaped morphology (Fig. 1A4). The mean intensity of $GFAP^+$ immunoreactivity in the hippocampus measured by confocal microscope was 45.9±4.8 (Fig. 5).

2.2. *Sham-operated genistein-treated rats*

Compared with the sham-operated animals, the following was found for the sham-operated animals that were given genistein: normal morphology in cresyl-violet staining, equivalent mean numbers of neurons/section (CA1, 150±4; CA3, 105±3; DGlb, 288±11; Figs. 2A–C), and the same patterns of Aβ, iNOS, nNOS, and GFAP immunoreactivity. For the number of $iNOS^+$ and $nNOS^+$ cells in the hippocampus see Figs. 4A–B.

2.3. *Aβ-injected rats*

Cresyl-violet-stained sections from the hippocampus of Aβ-injected rats differed from those obtained from sham-operated animals (Fig. 1B1). A few cells with intracellular brown pigment were observed along the DGlb and CC, and these were also recognized in unstained sections due to their brownish appearance. Sections from five of the six rats had homogeneous extracellular pink material close to the DGlb (Fig. 6A), and this material exhibited positive $A\beta_{1-40}$ immunoreactivity (Figs. 1B2 and 6B). The DGlb showed signs of extensive cell loss, which had developed mediolaterally and caused complete degeneration of this area in the studied sections from the five animals that exhibited Aβ-positive deposition. Furthermore, sparsely distributed pyramidal cells were seen in a short segment of the medial CA1. The numbers of neurons counted in CA1 (131±6; $P=0.03$), CA3 (79±7; $P=0.002$), and DGlb (35±11; $P<0.0001$) were significantly lower than the numbers found in the corresponding areas in the sham-operated rats (Figs. 2A–C).

The $iNOS^+$ (Fig. 3B1) and $nNOS^+$ (Fig. 3B2) neurons of the Aβ-injected rats showed more extensive intracellular