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Research Report

Inhibition of amyloid precursor protein secretases reduces recovery after spinal cord injury

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ABSTRACT

Amyloid-β (Aβ) is produced through the enzymatic cleavage of amyloid precursor protein (APP) by β (Bace1) and γ-secretases. The accumulation and aggregation of Aβ as amyloid plaques is the hallmark pathology of Alzheimer's disease and has been found in other neurological disorders, such as traumatic brain injury and multiple sclerosis. Although the role of Aβ after injury is not well understood, several studies have reported a negative correlation between Aβ formation and functional outcome. In this study we show that levels of APP, the enzymes cleaving APP (Bace1 and γ-secretase), and Aβ are significantly increased from 1 to 3 days after impact spinal cord injury (SCI) in mice. To determine the role of Aβ after SCI, we reduced or inhibited Aβ in vivo through pharmacological (using DAPT) or genetic (*Bace1* knockout mice) approaches. We found that these interventions significantly impaired functional recovery as evaluated by white matter sparing and behavioral testing. These data are consistent with a beneficial role for Aβ after SCI.

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1. Introduction

Amyloid-β (Aβ) is the product of the sequential cleavage of amyloid precursor protein (APP) by β-(Bace-1) and γ-secretases (Hu et al., 2006; Selkoe, 2004). Considerable data support a pathophysiological role for Aβ in Alzheimer's disease. Increases in APP, Bace-1, γ-secretase, and Aβ have also been observed with the onset of several other neurological disorders. Amyotrophic lateral sclerosis (ALS) onset is accompanied by an increase in APP, and elevated levels of both APP and Aβ have been observed in motor neurons and their surrounding glial cells in the spinal cord of ALS mouse models (Bryson et al., 2012). Patients with

Abbreviations: Aβ, amyloid-β; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; BLG, Basso mouse scale/Ladder climb/Grid walk; BMS, Basso mouse scale; DAPT, N-[(3, 5-Difluorophenyl)acetyl]-L-alanyl-2-phenylglycine-1,1-dimethylethyl ester; DEA, diethylamine; DPI, days post-injury; EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; SCI, spinal cord injury; TBI, traumatic brain injury

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multiple sclerosis (MS) show APP up-regulation in both acute and chronic lesions (Ikonomovic et al., 2004) and APP expression has been suggested as a biomarker for disease progression (Gehrmann et al., 1995). Aβ has also been implicated in microglial activation and associated neuroinflammation (Matsuoka et al., 2001; Wyss-Coray and Mucke, 2002).

In postmortem studies, Aβ deposits have been observed following traumatic brain injury (TBI) (Roberts et al., 1991; Roberts et al., 1994). Studies on excised surgery tissues show that Aβ accumulation is occurring in as little as two hours (Ikonomovic et al., 2004). The accumulation of Aβ also occurs in animal models of TBI (Iwata et al., 2002; Uryu et al., 2002). APP, BACE1, and PS1 are increased in the areas of axonal injury as early as 2 h after TBI (Chen et al., 2004; Uryu et al., 2007). Elevated levels of Aβ positively correlate with increases in β- and γ-secretases (Blasko et al., 2004). We recently demonstrated that inhibiting the activity of APP secretases and Aβ is protective in a mouse model of TBI (Loane et al., 2009), suggesting that Aβ may be contributing to secondary injury cascades after TBI. APP is also increased after spinal cord injury (SCI), in both humans and rodents (Ahlgren et al., 1996; Choo et al., 2008; Cornish et al., 2000; Li et al., 1995). A recent study in rats shows that the increase in APP and PS1 is accompanied by an increase in Aβ peptide levels as early as 1 day after spinal cord hemisection. This increase appears to occur within the axons in the white matter at the site of injury (Kobayashi et al., 2010). However, the injury-induced changes in APP secretases are not limited to neurons. After brain trauma, an increase in Bace1 and PS1 was reported in astrocytes (Blasko et al., 2004) and in both astrocytes and microglial cells, respectively (Nadler et al., 2008).

Although Aβ is implicated as a pathobiological factor in several neurological disorders, recent studies suggest that it may also have a protective role. Using a mouse model of MS – experimental autoimmune encephalomyelitis (EAE) – investigators demonstrated that Aβ peptide injections decreased paralysis and brain inflammation by suppressing activated lymphocytes (Grant et al., 2012). *Bace1* knockout (*Bace1* KO) mice, which do not produce Aβ, (Cai et al., 2001; Lu et al., 2000) show increased motor and cognitive deficits after TBI in the KO mice as compared to wild type controls (Mannix et al., 2011). To address the role of Aβ after SCI, we used a mouse spinal contusion model to examine effects of injury on APP, PS1, Bace1, and Aβ production. We also reduced Aβ formation after SCI using the γ-secretase inhibitor DAPT (N-[(3, 5-Difluorophenyl) acetyl]-L-alanyl-2-phenylglycine-1,1-dimethylethyl ester) or *Bace1* KO mice.

2. Results

2.1. SCI increases APP, PS1, and Bace-1

To study the expression of APP and PS1 before and after SCI, mice were sacrificed as sham or at 1, 3 and 7 days after moderate-severe injury ($n=4$/group). Fig. 1A shows sections from sham and 1, 3, and 7 day post injury (DPI) at the epicenter. Regions identified by asterisk and arrowhead are magnified in Fig. 1B. In sham, APP and PS1 are co-localized in the motor neurons in gray matter and the glial cells present in both white and gray matter. PS1 and APP increase at 1 and 3 days after injury, especially in the white matter, and return toward baseline by 7 DPI. APP and PS1 co-localize more after SCI injury as evident by the increased overlap of red and green in the merged images as compared to sham. Fig. 1C is a representative high resolution confocal image (taken from the area indicated in the thumbnail image by arrowhead) showing that some of the Iba1+ microglia express PS1 at 1 DPI.

Sections 1 mm and 2 mm rostral and caudal from the epicenter were evaluated from sham and injured mice ($n=3$/group) at 1, 3 and 7 days after injury using PS1. Fig. 2A shows a representative image and Fig. 2B summarizes the quantitative data. The thumbnail image represents the negative control for PS1. There is a significant increase (p-value <0.02), in PS1 protein 1 and 3 days after injury at the epicenter, as well as 1 mm rostral (p-value <0.001) and caudal (p-value <0.005) from the injury site. At 2 mm rostral (p-value <0.0001) and caudal (p-value <0.04) to the epicenter, a significant increase is only observed at 1 day after injury. The increase of PS1 in injured tissue compared to sham was confirmed using Western blots (Fig. 2C); PS1 protein levels are significantly increased (p-value <0.05) at 1 and 3 days after injury. Fig. 2D indicates that Bace1 protein levels are also significantly increased (p-value <0.05) at 1 DPI.

2.2. SCI acutely increases Aβ accumulation

To determine whether the increase in APP and its secretases is functionally relevant, mice ($n=4$/group) were injured and sacrificed at 1, 3, 7, 14, 21, and 28 days after SCI. $Aβ_{40}$ ELISA was performed on the diethylamine (DEA) extraction and Aβ was measured as fmol/mg protein (Fig. 3). There are no significant differences in Aβ levels between naive mice and sham mice euthanized 3 days post-surgery ($n=4$/group) indicating that laminectomy alone did not cause $Aβ_{40}$ up-regulation. Aβ is significantly increased (p-value <0.01) at 1 and 3 days after SCI compared to sham or naive samples. The increase in Aβ is concurrent with the increase in APP and its secretases.

2.3. DAPT significantly reduces Aβ accumulation after SCI

In order to confirm that the γ-secretase inhibitor DAPT can reduce the production of Aβ after SCI, C57/Bl6 spinal cord injured and sham mice ($n=4$/group) were administered either DAPT or vehicle as described in Section 4. Similar to our earlier experiment, SCI caused a significant increase in Aβ levels compared to sham injured mice. DAPT significantly (p-value <0.01) attenuated the SCI-induced increase in Aβ at 3d post-injury by 30% (Fig. 4).

2.4. DAPT administration reduces functional recovery after SCI in mice as measured by BMS and BLG

To investigate the effects of DAPT on functional recovery, C57/Bl6 injured mice ($n=12$/group) were administered either DAPT (30 mg/kg) or vehicle orally twice a day starting 15 min

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