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Collagen VI protects neurons against A β toxicity

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Abstract

Amyloid- β (A β) peptides, widely presumed to cause Alzheimer's disease, increased mouse neuronal expression of collagen VI through a mechanism involving transforming growth factor signaling. Reduction of collagen VI augmented A β neurotoxicity, whereas treatment of neurons with soluble collagen VI blocked the association of A β oligomers with neurons, enhanced A β aggregation and prevented neurotoxicity. These results identify collagen VI as an important component of the neuronal injury response and demonstrate its neuroprotective potential.

Introduction

Amyloid- β (A β) peptides are important in the pathogenesis of Alzheimer's disease. They are derived from amyloid precursor proteins (APP) and can self-aggregate into fibrillar and nonfibrillar structures. In transgenic mice with neuronal expression of familial Alzheimer's disease-mutant human APP (hAPP) and high A β levels, memory deficits and neuronal dysfunction are most directly related to soluble A β 1–42 (A β 42) oligomers¹. Many molecular alterations that correlate with memory deficits in these models are most prominent in the dentate gyrus, which is critical for learning and memory and shows early synaptic deficits in Alzheimer's disease².

We profiled A β -induced gene-expression changes in the dentate gyrus of hAPP transgenic mice and nontransgenic controls by DNA microarray analysis. All animal experiments were approved by the Institutional Animal Care and Use Committee of the University of California, San Francisco. Expression of mRNA encoding the α 1 subunit of collagen VI (*Col6a1*) was markedly increased in hAPP mice (data not shown). Collagen VI is a large triple-helix

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Author Contributions

J.S.C. and D.B.D. conducted the experiments and wrote the manuscript. J.L. performed atomic force microscopy analysis. I.H.C. prepared oligomeric A β . I.T., T.W.-C. and P.B. provided animal models and helpful discussions. D.H.K. and G.-Q.Y. provided technical assistance. L.M. supervised the project.

extracellular matrix (ECM) protein consisting of $\alpha 1(\text{VI})$, $\alpha 2(\text{VI})$ and $\alpha 3(\text{VI})$ subunits. Various forms of collagen have well-established roles in peripheral organs, but little is known about their functions in the brain. We are unaware of reports demonstrating activities of neuronal collagen VI.

We analyzed hippocampal *Col6a1* expression in nontransgenic and hAPP mice (lines J20 (refs. 1-3) and J9 (ref. 4)) and humans with or without Alzheimer's disease by quantitative RT-PCR and western blotting. At 3–6 months of age, *Col6a1* mRNA and $\alpha 1(\text{VI})$ protein levels in dentate gyrus were higher in hAPP-J20 mice than in nontransgenic controls (Fig. 1a,b). Additional hAPP-J20 mice, aged 2–18 months, also had higher hippocampal levels of *Col6a1* mRNA (Supplementary Fig. 1 online). In hAPP-J9 mice, which express about half as much hAPP/A β as hAPP-J20 mice⁴, hippocampal *Col6a1* mRNA levels were elevated, but to a lesser extent (Supplementary Fig. 1). In humans, *Col6a1* mRNA levels in dentate gyrus were higher in individuals with Alzheimer's disease than in nondemented controls (Fig. 1c). Humans with Alzheimer's disease and hAPP-J20 mice also showed higher dentate levels of *Col6a2* mRNA and a trend toward increased expression of *Col6a3* (Supplementary Fig. 1).

In situ hybridization to identify the cellular source of *Col6a1* revealed marked increases in expression in dentate gyrus granule cells in most hAPP-J20 mice (Fig. 1d). Less prominent increases were identified in the pyramidal layer of hippocampal subregions CA1 and CA3 and in the neocortex (Fig. 1d and data not shown).

To determine whether A β increases the expression of *Col6a1* mRNA and $\alpha 1(\text{VI})$ protein in neurons, we incubated primary hippocampal and cortical neurons from nontransgenic mice with synthetic human A $\beta 42$ oligomers (Supplementary Fig. 2 online) or a control peptide with a reversed sequence (A β rev). A $\beta 42$, but not A β rev, increased neuronal *Col6a1* mRNA and $\alpha 1(\text{VI})$ protein levels in a dose-dependent manner (Fig. 1e,f). Because A $\beta 5$ and collagen I⁶ bind APP, we tested primary neuronal cultures from APP-deficient mice. A β increased $\alpha 1(\text{VI})$ expression in these cultures (Fig. 1g), indicating that APP-dependent signaling is not critical in this process.

Because astrocytes participate in clearing extracellular A β and contribute to ECM production and remodeling⁷, we tested their response to A $\beta 42$. A $\beta 42$ did not affect $\alpha 1(\text{VI})$ expression in primary astrocyte cultures (Fig. 1h), but it did increase $\alpha 1(\text{VI})$ expression in mixed neuronal/glial cultures (Fig. 1i). Thus, A $\beta 42$ has neuron-specific effects on $\alpha 1(\text{VI})$ expression that are probably independent of astroglial activities.

To determine how A β regulates $\alpha 1(\text{VI})$ expression, we focused on a Smad3-responsive element in the *Col6a1* promoter region, identified in fibroblasts⁸. The transcription factor Smad3 regulates gene expression that is triggered by the injury-responsive cytokine transforming growth factor (TGF- β)⁹. Hippocampal TGF- β levels were increased in hAPP-J20 mice (Fig. 1j). In primary neuronal cultures, TGF- $\beta 1$ increased *Col6a1* expression (Fig. 1k). To determine whether TGF- β signaling mediates this effect, we treated primary neurons with a Smad3 inhibitor. Preventing Smad3 activation blocked the A $\beta 42$ -induced increase in $\alpha 1(\text{VI})$ expression (Fig. 1l). Thus, Smad3 may regulate $\alpha 1(\text{VI})$ in neurons as well as in fibroblasts⁸, and the A $\beta 42$ -induced increase in neuronal $\alpha 1(\text{VI})$ expression may be mediated by TGF- β and related signaling pathways.

Next, we focused on the TGF- β type II receptor (T β RII), which regulates Smad3 activation⁹ and can modulate A β toxicity¹⁰. We tested the effect of A $\beta 42$ on primary neuronal cultures from conditional knockout mice lacking T β RII expression in forebrain neurons. Unlike wild-type neurons, T β RII-deficient neurons failed to increase $\alpha 1(\text{VI})$ expression in response to A $\beta 42$ oligomers (Fig. 1m). Taken together, these results suggest that A $\beta 42$ -induced increases in neuronal $\alpha 1(\text{VI})$ expression involve T β RII-dependent activation of Smad3.

What functions may collagen VI fulfill in the nervous system? Because collagen VI inhibits apoptosis in cells of peripheral organs¹¹, we tested whether it increases neuronal resistance to A β neurotoxicity by analyzing primary neuronal cultures from $\alpha 1(VI)$ -deficient mice, which cannot produce collagen VI (ref. 12). Although $\alpha 1(VI)$ ablation did not diminish the survival of untreated cells, wild-type neurons were more resistant to A β 42 oligomers than $\alpha 1(VI)$ -deficient neurons (Fig. 2a).

We also examined the effect of purified collagen VI on A β -induced neurotoxicity more directly. The structure and assembly of collagen are highly dependent on pH¹³. Fibrillar collagen VI was abundant at pH 7–8, whereas nonfibrillar, denatured species predominated at pH 2–3 (Fig. 2b). Treatment of wild-type and $\alpha 1(VI)$ -deficient (data not shown) neurons with collagen VI at pH 7–8 prevented A β 42-induced cell death in a dose-dependent manner (Fig. 2c). Denatured collagen VI and fibrillar collagen I were not protective (Fig. 2d,e).

To explore the mechanism of this protection, we examined whether collagen VI alters the interaction of A β 42 oligomers with neurons in culture. In the absence of collagen VI, A β 42 oligomers were closely associated with neurons (Fig. 2f), probably reflecting binding of A β 42 to neuronal surfaces, neuronal uptake of A β 42 oligomers, or both^{14, 15}. Notably, collagen VI blocked the association of A β 42 oligomers with immature (Fig. 2f) and mature (Fig. 2g) neurons, whereas collagen I did not (Fig. 2g and data not shown). In the presence of collagen VI, A β 42 oligomers were sequestered into large aggregates that colocalized with collagen VI in the extracellular milieu (Fig. 2h). Atomic force microscopy showed that collagen VI promoted the assembly of A β 42 into larger aggregates (Fig. 2i,j), resulting in fewer potentially neurotoxic A β oligomers (Fig. 2k). Thus, collagen VI may prevent A β 42 neurotoxicity, at least in part, by promoting the extracellular sequestration of A β 42 oligomers and preventing their interaction with neurons.

Our findings reveal previously unknown actions of collagen VI in the nervous system and suggest that increased collagen VI expression in the brains of hAPP mice and individuals with Alzheimer's disease is a neuroprotective response. It is unlikely that this response is completely specific to A β 42-induced injury, as hydrogen peroxide also increased *Col6a1* expression in primary neurons, albeit to lesser extent than A β 42 (data not shown). A β 42 oligomers increased $\alpha 1(VI)$ expression in neurons by an astrocyte-independent mechanism involving T β RII and Smad3 activation. TGF- β can promote apoptosis under specific circumstances, but also fulfills many beneficial functions in injury responses and ECM organization. Because hAPP-J20 mice had increased hippocampal levels of TGF- β , this cytokine may mediate the effect of A β 42 on neuronal collagen VI expression. However, we cannot exclude that another factor or even A β itself binds to T β RII, activating T β RII-dependent signaling and gene expression.

The ability of collagen VI to block the interaction between A β 42 oligomers and neurons is a plausible mechanism for its neuroprotective effect and might be instructive in the development of new therapeutic strategies for treating Alzheimer's disease. Additional mechanisms cannot be excluded and merit further investigation.

Supplementary Material

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Figure 1. Aβ increases neuronal expression of the collagen VI α1 subunit through mechanisms involving TGFβ signaling

(a–c) Dentate gyrus *Col6a1* mRNA (a) and α1(VI) protein (b) levels in nontransgenic (NTG) and hAPP-J20 mice (n = 11–13 per group, aged 3–6 months) and dentate gyrus *Col6a1* mRNA levels (c) in nondemented humans (CTL, n = 3) and individuals with Alzheimer's disease (AD, n = 8) (Supplementary Methods and Supplementary Fig. 3 online). The mean *Col6a1*/Gapdh mRNA, α1(VI)/tubulin and *Col6a1*/NSE mRNA ratios in nontransgenic or CTL samples (a–c) were defined as 1. (d) In situ hybridization of coronal mouse brain sections with a *Col6a1*-specific antisense riboprobe. Scale bars represent 500 μm. (e,f) Primary cultures of purified neocortical/hippocampal neurons of nontransgenic mice treated for 24 h with vehicle (Veh), Aβ or Aβrev (45 or 90 μg ml⁻¹). *Col6a1* mRNA levels in cell lysates (e) and α1(VI) protein levels in medium (f, representative western blot signals above). The average *Col6a1*/Gapdh mRNA and α1(VI)/tubulin protein ratios in vehicle-treated cultures were defined as 1. (g) α1(VI) protein levels in medium of neuronal cultures from APP-deficient mice treated for 24 h with vehicle, Aβ42 (90 μg ml⁻¹) or Aβrev (90 μg ml⁻¹). (h,i) α1(VI) protein levels in medium

of nontransgenic cultures of mouse astrocytes (h) or neurons and astrocytes (i) treated with vehicle, A β 42 or A β rev for 24 h. (j) Hippocampal levels of bioactive TGF β in 6–7-month-old nontransgenic (n = 7) and hAPP-J20 (n = 5) mice, measured by bioassay. (k) *Col6a1* mRNA levels in nontransgenic neuronal cultures treated with vehicle or bioactive TGF β 1 (10 nM) for 24 h. (l) Nontransgenic neuronal cultures treated with culture medium (Veh), dimethyl sulfoxide (DMSO, 0.2% final concentration, vol/vol) or A β peptides (90 μ g ml⁻¹). Cultures were treated with a Smad3 inhibitor (SIS3) 1 h before and throughout the 24 h exposure to A β and α 1(VI) levels were measured in the medium. (m) Purified neuronal cultures from T β RII wild-type (+) mice or knockout mice (–) lacking T β RII expression in forebrain neurons were treated with vehicle or A β peptides (90 μ g ml⁻¹), and α 1(VI) levels were measured in the medium. For all cell culture studies, n = 4–8 wells per condition from 2–3 independent experiments. *P < 0.05, **P < 0.01 and ***P < 0.001 versus leftmost bar or as indicated by bracket (Tukey test). Error bars represent s.e.m.

Figure 2. Collagen VI blocks neuronal association and neurotoxicity of Aβ₄₂ oligomers

(a) Primary cultures of purified neocortical/hippocampal neurons from mice with various *Col6a1* genotypes treated with Aβ₄₂ or Aβ_{rev} (90 μg ml⁻¹) for 24 h. Neurotoxicity was determined by trypan blue staining. (b) Ex situ atomic force microscopy images of fibrillar (pH 7–8) and denatured (pH 2–3) collagen VI. Scale bar represents 200 nm. (c–e) Beginning 1 h before and lasting throughout exposure to Aβ (45 μg ml⁻¹), wild-type neuronal cultures were treated with vehicle, human collagen VI at pH 7–8 (0–100 μg ml⁻¹, c) or pH 2–3 (20 μg ml⁻¹, d), or human collagen I at pH 7–8 (20 μg ml⁻¹, e). *n* = 4–8 wells/condition in 1–2 independent experiments. (f) Wild-type neuronal cultures were pretreated with vehicle or collagen VI (pH 7–8) and exposed to Aβ₄₂ (45 μg ml⁻¹). After 1 h, the cell association of Aβ₄₂ oligomers was assessed by immunostaining (6E10 antibody). Neuronal nuclei were stained (DAPI, blue). In vehicle-treated cultures, most Aβ was cell associated. In collagen VI-treated cultures, most Aβ was sequestered in large extracellular aggregates. Scale bar represents 10 μm. (g) Mature wild-type neurons (4 weeks) pretreated with vehicle, collagen VI (pH 7–8) or collagen I (pH 7–8) were exposed to Aβ₄₂ (45 μg ml⁻¹) and immunostained for Aβ (red) and microtubule-associated protein 2 (MAP-2, green). Scale bar represents 20 μm. (h) Immature neurons were treated with collagen VI and Aβ₄₂ (as in f and g) and double labeled for Aβ (red) and collagen VI (green). Scale bar represents 20 μm. We observed similar immunostaining patterns in three independent experiments per condition. (i) Aggregation of monomeric Aβ₄₂ was monitored by in situ AFM for 3 h. Images show the aggregation state 145 min after incubation of Aβ₄₂ in the absence (top) or presence (bottom) of collagen VI (pH 7–8). Scale bar represents 2 μm. (j,k) Large Aβ aggregates (greater than or equal to 40 times 103 nm², representing >95th percentile in size of all Aβ aggregates, j) and small Aβ aggregates (less than or equal to 4 times 103 nm², estimated to consist of <40 Aβ peptides, k) visualized by AFM were quantified at 145 min (*n* = 4 images per condition). AFM results were repeated in two independent experiments. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 versus leftmost bar or as indicated by bracket (Tukey test or Fisher's exact test). Error bars represent s.e.m.