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Perlecan domain V inhibits α2 integrin-mediated amyloid-β neurotoxicity

Sarah Wright[a,1], Christi Parham[b], Boyeon Lee[b], Douglas Clarke[b], Lisa Auckland[b], Jennifer Johnston[a], Andrea L. Lawrence[c], S. Kent Dickeson[c], Samuel A. Santoro[c], Irene Griswold-Prenner[a,1], Gregory Bix[b,*]

[a] *Elan Pharmaceuticals Inc., South San Francisco, CA, USA*

[b] *Department of Molecular and Cellular Medicine and Department of Neuroscience and Experimental Therapeutics, Texas A&M, College Station, TX, USA*

[c] *Department of Pathology, Vanderbilt University School of Medicine, Nashville, TN, USA*

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Abstract

Amyloid-β (Aβ) peptide is a key component of amyloid plaques, one of the pathological features of Alzheimer's disease. Another feature is pronounced cell loss in the brain leading to an enlargement of the ventricular area and a decrease in brain weight and volume. Aβ plaque deposition and neuronal toxicity can be modeled by treating human cortical neuronal cultures with Aβ and showing robust Aβ deposition and neurotoxicity that is mediated by α2β1 and αvβ1 integrins. The current study expands on these findings by showing that the domain V of perlecan, a known α2 integrin ligand, inhibits Aβ neurotoxicity in an α2 integrin-dependent manner. Additionally, Aβ binds more efficiently to cells expressing activated α2 integrin. Finally the inhibition of Aβ neurotoxicity with domain V is synergistic with inhibitors of αv integrin and β1 integrin. We propose that domain V and potentially other α2 integrin ligands could be a new therapeutic approach for inhibiting the Aβ plaque deposition and neurotoxicity observed in Alzheimer's disease.

Keywords: Amyloid-β; Alzheimer's disease; Domain V; Perlecan; α2β1 integrin; Neurodegeneration

1. Introduction

The loss of cells and profound brain atrophy in Alzheimer's disease (AD) no doubt contributes to many of the disease's clinical features. The mechanism by which the cells are lost is unknown, although some have proposed an initial synaptic toxicity which is followed by overt cell death (Delaere et al., 1989). The mechanism of the initial synaptic toxicity is an area of intense research. Amyloid-β (Aβ) peptide, the primary component of the Alzheimer's disease plaque, has been suggested to play a role in both synaptic and overt cell toxicity, beginning with the observation of Lue (Lue et al., 1999) that soluble Aβ, and not plaque-associated Aβ, correlated with loss of the synaptic protein synaptophysin. Additional work by many investigators has shown that nonfibrillar forms of Aβ can have some synaptotoxic (Lacor et al., 2004; Wang et al., 2004) or overt toxic effects in cultures (Kim et al., 2003).

Our approach to understanding Aβ neurotoxicity has employed a polymerization dependent toxicity (Wogulis et al., 2005), whereby soluble and fibrillar Aβ is combined to allow the material to aggregate. If the aggregation occurs in or on a cell membrane, robust toxicity and deposition occurs. This does not rule out the possibility of oligomers forming during the aggregation process, but toxicity is

* Corresponding author at: Department of Molecular and Cellular Medicine and Department of Neuroscience and Experimental Therapeutics, 426 Joe H. Reynolds Medical Building, Texas A&M, College Station, TX 77843, USA. Tel.: +1 979 862 7613; fax: +1 979 847 9481.

E-mail address: GJBix@medicine.tamhsc.edu (G. Bix).

[1] Current address: iPierian, 951 Gateway Boulevard, South San Francisco, CA 94080, USA.

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clearly dependent upon aggregation occurring in the presence of neurons. Using this system we previously identified specific integrin heterodimers that appear to mediate both deposition and overt toxicity (Wright et al., 2007) as well as synaptic toxicity, as measured by Aβ effects on long term potentiation (LTP) (Wang et al., 2008).

Domain V (DV) is the 85 kDa C-terminal domain of the heparan sulfate proteoglycan, perlecan. Perlecan itself plays a significant role in key cellular processes, including roles in cell growth, differentiation, brain and cardiovascular development, and inflammation, and is a prominent component of the vascular basement membrane throughout the body (Bix and Iozzo, 2008). Perlecan is composed of 5 domains, each with structural homology to other proteins. DV consists of 3 laminin globular (LG) domains, each separated by 2 epidermal growth factor (EGF)-like domains. DV and specifically, its C-terminal LG3 domain, is proteolyzed from full length perlecan by the bone morphogenetic protein 1 (BMP-1) family of tolloid metalloproteases (Gonzalez et al., 2005), cathepsin L (Cailhier et al., 2008), and is normally found in the human blood and cerebrospinal fluid (CSF) proteomes as well as in the urine of patients with end stage renal disease (Adkins et al., 2002; Cartier et al., 2004; Pieper et al., 2004). Domain V and LG3 have been shown to inhibit angiogenesis in nonbrain endothelial cells in vitro and in vivo via direct interaction with the ligand binding domain (I domain) of α2 integrin, previously described (Bix et al., 2004., 2006).

Due to the interaction of DV and LG3 with α2 integrin, their presence in the brain and the requirement for α2 integrin for Aβ deposition and neurotoxicity, we hypothesized that these molecules might play a role in Aβ neurotoxicity.

In this study we further implicate α2β1 integrin in both binding to Aβ and mediating Aβ neurotoxicity by showing that Aβ binds to activated α2 integrin-expressing cells and DV and LG3 can protect from Aβ neurotoxicity in an α2 integrin-dependent manner.

2. Methods

2.1. Tissue culture

Human cortical (HCC) and mouse cortical (MCC) cultures from α2 null mice and wild-type littermate controls in a C57Bl6 background (kindly provided by Mary Zutter, Vanderbilt University, Nashville, TN, USA) were prepared as described in Wright et al. (2007). Human fetal cerebral cortical tissue was obtained by Advanced Bioscience Resources (Alameda, CA, USA) and complied with federal guidelines for fetal research and with the Uniformed Anatomical Gift Act.

2.2. Aβ preparation

Aβ (1–40) (lot ML0810, California Peptide Research, Napa, CA, USA) preparation and characterization was conducted as described previously (Wogulis et al., 2005). Briefly Aβ powder is made up as 2 solutions: (1) dissolved in dimethyl sulfoxide (DMSO) at 7.5 mM and sonicated (soluble preparation), and (2) dissolved in tissue culture grade sterile H_2O at 1 mM and aged for 3–8 days until gelled (aggregated preparation). Robust toxicity is generated over 2–3 days by treating cells for 1 hour with 1 μM aggregated solution followed by aspiration and addition of 15–20 μM of the soluble Aβ (Wogulis et al., 2005). This lot of Aβ (ML0810) generated toxicity using this 2 component method but was also found to generate equivalent toxicity when made up as a tissue culture grade sterile H_2O stock at 1 mM and immediately aliquotted and frozen (data not shown) due to an appropriate ratio of aggregated to soluble fractions. The same lot of Aβ (1–40) was used throughout these studies.

2.3. DV and LG3 preparation/purification

Human DV was cloned into the vector pSecTag2A (Invitrogen, Carlsbad, CA, USA) using the following primers: 5′ DV Asci pSecTag 2A: 5′ AGG GCG CGC CAT CAA GAT CAC CTT CCG GC 3′; 3′ DV Xho1 pSEc Tag 2A: 5′ AGC TCG AGC CGA GGG GCA GGG GCG TGT GTT G 3′. The DV complementary DNA (cDNA) was amplified from human umbilical vein endothelial cell (HUVEC) cDNA utilizing a GC-rich polymerase chain reaction (PCR) system and dNTPack (Roche Applied Science, Indianapolis, IN, USA) and used the restriction enzymes Xho1, Asc1 (NEB Corp., Ipswich, MA, USA). Maxi-preps of DV DNA were transfected into 293FT (ATCC, Manassas, VA, USA) cells via lipofectamine (Invitrogen). After transfection, the 293 cells were exposed to serum-free media for 48–72 hours followed by collection and DV purification via its C-terminal 6XHis tag and Ni-ATA agarose bead (Qiagen, Valencia, CA, USA) as per the company's instructions. Eluted fractions that contained DV were combined and dialyzed against 1 × phosphate buffered saline (PBS) and the purity of the resultant DV was confirmed via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) stained with Brilliant Blue G-colloidal and by Western blot analysis using commercially available anti-DV antibody (R&D Systems, Minneapolis, MN, USA) and anti-His antibody (EMD Chemicals, Gibbstown, NJ, USA). The DV was quantified with Quick Start Bradford Dye Reagent (Biorad, Hercules, CA, USA). The LG3 C-terminal domain of DV was cloned and ligated using the pCEP-PU vector (kindly provided by Maurizio Mongiat, The Cancer Institute, Aviano, Italy) using the following primers: 5′ AGG-CATACGCATGGCATAGCAATAGCAGAGTC-NHEI 3′; 3′ AGC TCG AGC ATGATG ATGATGATGATGC-GAGG-XHO1 3′. LG3 was expressed in 293FT cells and purified via an added C-terminal 6XHis tag in an identical fashion to DV. Activity of DV and LG3 was confirmed by HUVEC adhesion assays, performed as previously described (Mongiat et al., 2003, data not shown) in which DV