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Research report

Anti-angiogenic activity of the mutant Dutch Aβ peptide on human brain microvascular endothelial cells

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Abstract

Cerebral amyloid angiopathy is a common pathological feature of patients with Alzheimer's disease (AD) and it is also the hallmark of individuals with a rare autosomal dominant disorder known as hereditary cerebral hemorrhage with amyloidosis-Dutch type. We have shown previously that wild type Aβ peptides are anti-angiogenic both in vitro and in vivo and could contribute to the compromised cerebrovascular architecture observed in AD. In the present study, we investigated the potential anti-angiogenic activity of the Dutch $A\beta_{1-40}$ (E22Q) peptide. We show that compared to wild type Aβ, freshly solubilized Dutch Aβ peptide more potently inhibits the formation of capillary structures induced by plating human brain microvascular endothelial cells onto a reconstituted basement membrane. Aggregated/fibrillar preparations of wild type Aβ and Dutch Aβ do not appear to be anti-angiogenic in this assay. The stronger anti-angiogenic activity of the Dutch Aβ compared to wild type Aβ appears to be related to the increased formation of low molecular weight Aβ oligomers in the culture medium surrounding human brain microvascular endothelial cells. Using oligonucleotide microarray analysis of human brain microvascular endothelial cells, followed by a genome-scale computational analysis with the Ingenuity Pathways Knowledge Base, networks of genes affected by an anti-angiogenic dose of Dutch Aβ were identified. This analysis highlights that several biological networks involved in angiogenesis, tumorigenesis, atherosclerosis, cellular migration and proliferation are disrupted in human brain microvascular endothelial cells exposed to Dutch Aβ. Altogether, these data provide new molecular clues regarding the pathological activity of Dutch Aβ peptide in the cerebrovasculature.

Theme: Disorders of the nervous system
Topic: Degenerative disease: Alzheimer's-beta amyloid

Keywords: Angiogenesis; Amyloid; Oligomer; Cerebral amyloid angiopathy; Gene expression; Oligonucleotide microarray; Dutch mutation

1. Introduction

Alzheimer's disease (AD) is neuropathologically characterized by the progressive deposition of β-amyloid (Aβ) in the brain parenchyma and in cortical and leptomeningeal blood vessels [59]. Cerebral amyloid angiopathy (CAA) is a common pathological feature of patients with AD. Extensive CAA is also the hallmark of individuals with a rare autosomal dominant disorder known as hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D) [66]. HCHWA-D is caused by a point mutation at codon 693 of the β-amyloid precursor protein resulting in a glutamic acid to glutamine substitution at residue 22 (E22Q) of Aβ leading to recurrent and often fatal hemorrhagic episodes at mid-life [44]. The reason why the Dutch mutation leads to a strong accumulation of Aβ in the cerebrovasculature followed by events of stroke and cerebral hemorrhage remains unclear. However, it is known that the Dutch E22Q mutation results in loss of a negative charge in the Aβ peptide and enhances its fibrillogenic and pathogenic properties. In vitro, Dutch Aβ (E22Q) at a

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concentration of at least 25 μM (but not wild type Aβ at the same concentration) has been reported to be toxic, after 6–12 days of incubation with human smooth muscle cells [18,48,67,69]. In vivo, the presence of Aβ in the walls of cerebral blood vessels is closely associated with degeneration of smooth muscle cells [70,71]. A defect in reverse transport to the systemic circulation as shown for the Dutch Aβ variant together with inefficient local proteolysis may accelerate its rate of aggregation and deposition in the cerebrovasculature [4,49,51,64].

Numerous structural and functional cerebromicrovascular abnormalities have been identified in AD subjects, including reduction of the cerebral blood flow, decreased capillary diameter, thinning of capillary basement membrane, attenuation of capillary endothelium and cerebrovascular muscle atrophy [14,36,37,46]. In addition, capillary densities have been shown to be reduced in AD brains and Down's syndrome brains, markedly in areas of the brain affected by high Aβ burden [9,10,25,47,63] whereas other reports have shown no change in some specific areas of the brain in AD [7,8]. We have shown that wild type $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides exhibit a potent anti-angiogenic activity both in vitro and in vivo [55] which could contribute to the altered cerebrovascular architecture observed in AD cases. In the present report, we compared the anti-angiogenic activity of the Dutch $A\beta_{1-40}$ (E22Q) to wild type $A\beta_{1-40}$ on the formation of capillary structures in vitro using human brain microvascular endothelial cells. In order to delineate the molecular mechanisms involved in the anti-angiogenic activity of Dutch Aβ, we treated human brain microvascular endothelial cells with an anti-angiogenic dose of Dutch $A\beta_{1-40}$ and analyzed the effect of this treatment on endothelial genes expression using Affymetrix DNA microarrays. To increase the effectiveness of DNA microarray analysis, global gene expression data were combined with external data sources in order to associate the expression patterns of a set of genes with biological functions. We used the Ingenuity Pathways Knowledge Base to provide a genome-scale computational solution for determining the effect of Dutch $A\beta_{1-40}$ on human brain microvascular endothelial cells.

2. Material and methods

2.1. Aβ peptides

Wild type $A\beta_{1-40}$ and Dutch $A\beta_{1-40}$ (E22Q) with purity greater than 95% were purchased from Biosource, CA. Aβ peptides were first dissolved to 1 mM in cold (4 °C) hexafluoro–isopropanol (HFIP) in a chemical fume hood to break down β-sheet structure and disrupt hydrophobic forces in aggregated Aβ (that may be present in the lyophilized stock of peptides)[26] HFIP was allowed to evaporate in the fume hood and the resulting clear peptide films were dried under vacuum in a SpeedVac. HFIP treated peptides were completely dissolved to 5 mM in anhydrous dimethyl sulfoxide (DMSO) by pipette mixing.

2.2. Culture of human brain microvascular endothelial cells

Human brain microvascular endothelial cells were purchased from ScienCell, CA and grown in 75 cm^2 flasks (at 37 °C, 5% CO_2) containing endothelial cell medium (EC, ScienCell, CA) supplemented with 5% serum and with 1% endothelial cell growth supplement (ScienCell, CA), with the medium changed every 3 days.

2.3. Capillary morphogenesis assay

For the capillary network assay, 500 μL of human brain microvascular endothelial cells (density of 75,000 cells/mL; passage 3 to 4) diluted in Kaighn's F12K medium containing 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mg/mL heparin, 0.03 mg/mL endothelial cell growth supplement and 10% fetal bovine serum were plated in 24 well-plates previously coated with 250 μL of Matrigel according to the recommendation of the manufacturer (BD Bioscience, CA). Aβ peptides (HFIP treated and dissolved to 5 mM in DMSO as indicated above) were added to the cells (1, 5, 10 and 50 μM) at the time of plating on Matrigel (control wells were treated with the same volume of DMSO). Experiments were repeated using preparations of aggregated Dutch $A\beta_{1-40}$ and wild type $A\beta_{1-40}$. Dutch $A\beta_{1-40}$ and wild type $A\beta_{1-40}$, at a concentration of 100 μM in distilled water, were incubated at 37 °C for 7 days. Aggregated insoluble Aβ in these preparations was precipitated by centrifugation at 15,000 × *g* for 30 min, washed with 1 mL of PBS (to remove non aggregated Aβ species which tend to associate to aggregated Aβ, data not shown) and pelleted by centrifugation at 15,000 × *g* for 30 min. Aggregated insoluble material was then resuspended in PBS by vortexing and added to the endothelial cells (1, 5, 10 and 50 μM) at the time of plating on Matrigel. 6 Matrigel wells were used for each dose and form (freshly solubilized, aggregated) of wild type Aβ and Dutch Aβ. Following 24 h of incubation at 37 °C, 5% CO_2, 2 randomly chosen fields (4× magnification) from each Matrigel well were photographed under an inverted microscope with a digital camera. The length of the capillary network in each well was estimated by image analysis using the Image Pro Plus software (Media Cybernetic, LP) as previously described [55]. Results were expressed as a percentage of total capillary length measured in the control conditions. Multiple comparisons were evaluated by analysis of variance and post hoc comparisons performed with Bonferroni's method using SPSS V12.0.1 for Windows. Probability values less than 5% were considered statistically significant.

To analyze the different forms of Aβ present in the culture medium surrounding human brain microvascular endothelial cells, an aliquot (20 μL) of culture medium was collected from the culture wells treated with 1 and 5 μM of