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Novel phage peptides attenuate beta amyloid-42 catalysed hydrogen peroxide production and associated neurotoxicity

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Abstract

Amyloid-β (Aβ) peptides play a central role in the pathogenesis of Alzheimer's disease. There is accumulating evidence that supports the notion that the toxicity associated with human Aβ (both 40 and 42) is dependent on its superoxide dismutase (SOD)-like activity. We developed a novel screening method involving phage display technology to identify novel peptides capable of inhibiting Aβ's neurotoxicity. Two random peptide libraries containing 6-mer and 15-mer peptide inserts were used and resulted in the identification of 25 peptides that bound human Aβ (40 or 42). Here, we show that two of the three most enriched peptides obtained significantly reduced Aβ42's SOD-like activity. A 15-mer peptide reduced Aβ42 neurotoxicity in a dose-dependent manner as evidenced by a reduction in LDH release. These findings were confirmed in the independent MTT assay. Furthermore, comparative analysis of the 15-mer peptide with Clioquinol, a known inhibitor of Aβ's metal-mediated redox activity, showed the 15-mer peptide to be equipotent to this metal chelator, under the same experimental conditions. These agents represent novel peptides that selectively target and neutralise Aβ-induced neurotoxicity and thus provide promising leads for rational drug development.

Keywords: Phage display; Peptides; Beta amyloid; Neutralization; Drug development

1. Introduction

Alzheimer's disease (AD) is the most common form of cerebral degeneration leading to dementia, affecting an estimated 12 million people worldwide. The prevalence of this disease is expected to increase rapidly due to the overall growth and ageing of the world's population. Conservative estimates indicate there will be 22 million sufferers worldwide by 2025 (Access Economics Pty. Ltd., 2003). While a small number of therapeutic drugs are available for the treatment of AD, they only benefit some patients for a period of less than 2 years, in part because of side-effects, but mainly because they only address the cognitive symptoms, and not the underlying cause of the disease. In the past decade, FDA-approved drugs have been directed towards managing alterations in neurotransmitter, inflammatory, oxidative and

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hormonal pathways. Thus, there is an increasing urgency to develop more effective therapeutic agents for the treatment of AD.

AD can only be conclusively diagnosed by post-mortem examination of the brain for pathological hallmarks, including intracellular deposits known as neurofibrillary tangles and extracellular deposits called amyloid plaques. A major protein component of the plaques is beta amyloid (Aβ), a 4 kDa spontaneously aggregating peptide of 39–43 amino acids (Glenner and Wong, 1984; Masters et al., 1985). Aβ is produced by the sequential proteolytic cleavage of its parent transmembrane protein—the amyloid precursor protein (APP) (Kang et al., 1987) by two enzymes known as BACE and γ-secretase (for a review, see Verdile et al., 2004). Cerebral Aβ levels increase in both sporadic and familial AD, where the levels can be directly influenced by both single gene defects in the APP or presenilin genes, and/or the interaction of multiple environmental and genetic risk factors (Rogaev et al., 1995).

Aβ is a naturally occurring neuropeptide that exists in a number of forms. The monomeric form is soluble; however, Aβ can aggregate readily, in a process known as fibrillization, to form dimers and polymers of increasing size. While all forms have been implicated as the potential toxic agent in AD and induce neurotoxicity by distinct mechanisms (Deshpande et al., 2006), disease progression has been shown to correlate more closely with the level of soluble Aβ oligomers, and there is considerable evidence in the literature to support these Aβ oligomers as the principal toxic species (for a review, see Walsh and Selkoe, 2007). Redox-active transition metals have been shown to accelerate aggregation of the Aβ peptide (Atwood et al., 1998; Mantyh et al., 1993). The solubility of Aβ decreases with increasing aggregation, leading to the deposition of the extracellular insoluble amyloid fibrils and plaques. Of critical importance is the fact that in all known inheritable early-onset forms of the disease, the genetic mutations have been shown to increase either the levels of Aβ, or to increase the levels of the more insoluble longer forms, Aβ1–42/43. This implies that an increase or an imbalance in Aβ production is enough to initiate disease development.

The toxicity of the Aβ peptide is thought to be responsible for the progressive cognitive decline associated with AD. While the exact manner by which Aβ exerts its toxicity upon the central nervous system remains unclear, several mechanisms have been proposed. These include the formation of dityrosine Aβ oligomers via a mechanism involving metal-catalysed redox chemistry (Barnham et al., 2004); and the interaction of Aβ with lipid membranes which results in changes in membrane fluidity, leading to depolarization and disorder (Müller et al., 1995), formation of specific channels (pores) that could affect calcium homeostasis (Kawahara and Kuroda, 2000), and lipid peroxidation via membrane-associated free radical formation (Butterfield et al., 2001) and cholesterol oxidation (Puglielli et al., 2005).

A large body of evidence exists to show that the interaction of biometals with Aβ plays an important role in the pathogenesis of AD (for a review, see Bush, 2003). Evidence includes: increased levels of biometals, such as copper (Cu^{2+}), zinc (Zn^{2+}) and iron (Fe^{3+}) are found in Aβ plaques (Lovell et al., 1998); copper and zinc control the aggregation state of Aβ; and Aβ-Cu^{2+} complexes are redox active that result in free radical generation (Bush, 2003). Interestingly, the Aβ25–35 fragment of full length Aβ does not contain the direct metal binding sites (histidines 6, 13, 14 and tyrosine 10), yet it still aggregates and demonstrates neurotoxicity. However, it should be noted that the Aβ25–35 fragment is not found naturally and that its mechanism of neurotoxicity may differ to that seen for full length Aβ. Aβ itself may be subject to attack by free radicals, where the presence of Cu^{2+} can catalyse oxygenation, mainly at the methionine 35 (Met35) sulphur atom (Nishino and Nishida, 2001) and dityrosine formation (Atwood et al., 2004). In addition, it has recently been reported that copper can bind Aβ via the N-terminal amino group (Syme et al., 2004), or an as yet unidentified carboxylate side chain (Karr et al., 2004). While the Met35 residue of Aβ has been reported to be critical for neurotoxicity, aggregation, and free radical/reactive oxygen species formation, experiments using Aβ without methionine, such as AβM35V (methionine to valine substitution at position 35) have shown toxicity. This is believed to be due to increased lipid membrane binding as shown by EPR spectroscopy (Ciccotosto et al., 2004). Hence, it is possible that there is more than one mechanism by which Aβ exerts neurotoxicity.

The accumulation of Aβ is associated with a number of markers of oxidative stress including protein oxidation (Smith et al., 1997), lipid peroxidation (Mark et al., 1997; Sayre et al., 1997), and oxidation of nucleic acids (Nunomura et al., 1999). Aβ accumulation in cultured neuronal cells leads to an increase in hydrogen peroxide (H_2O_2) levels (Behl et al., 1994) and the production of reactive oxygen intermediates (Harris et al., 1995). More recently, the single methionine residue at position 35 of Aβ, possibly in conjunction with redox metal ions bound near the N-terminus, has been shown to be critical for the oxidative and neurotoxic properties of this peptide (Butterfield and Bush, 2004; Yatin et al., 1999). This is just some of the evidence underlying the theory that Aβ plays a key role in oxidative stress-evoked neuropathology. This process of oxidative stress is believed to contribute to a breakdown of synaptic function in neurons, and ultimately neuronal cell death, leading to the progressive permanent loss of cognitive function which is a characteristic feature of AD.

Superoxide dismutases (SOD) are metalloenzymes containing a redox-active transition metal [copper (Cu), iron (Fe) or manganese (Mn)] active site. SOD catalyses the reduction of superoxide radicals to H_2O_2 by electron transfer between the superoxide anions and the transition-active metal. While low levels of SOD enzymes do provide some protection by detoxifying superoxide radicals thereby preventing superoxide mediated cell damage, high levels of these enzymes