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MRZ-99030 – A novel modulator of Aβ aggregation: I – Mechanism of action (MoA) underlying the potential neuroprotective treatment of Alzheimer's disease, glaucoma and age-related macular degeneration (AMD)

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ABSTRACT

Therapeutic approaches addressing β-amyloid$_{1-42}$ (Aβ$_{1-42}$) aggregation represent a promising neuroprotective strategy for the treatment of Alzheimer's disease, dry age-related macular degeneration (AMD) and glaucoma. MRZ-99030 is a dipeptide containing D-tryptophan and 2-amino-2-methylpropionic acid in clinical development for the topical treatment of glaucoma and AMD.

MRZ-99030 is an Aβ aggregation modulator, previously reported to prevent the formation of soluble toxic oligomeric Aβ species. The present study confirmed that MRZ-99030 prevents the formation of oligomeric Aβ species using similar SDS-PAGE experiments. However, additional data from TR-FRET, DLS and AFM experiments revealed that MRZ-99030 does not directly prevent early protein/protein interactions between monomeric Aβ, but rather promotes the formation of large, non-amyloidogenic, amorphous Aβ aggregates and thereby reduces the amount of intermediate toxic soluble oligomeric Aβ species.

The affinity of MRZ-99030 to Aβ$_{1-42}$ determined by SPR was 28.4 nM but the ratio of compound to Aβ is also important: a 10–20 fold excess of MRZ-99030 over Aβ is probably required for effective inhibition of protein/protein interactions. For example, in glaucoma, assuming a maximal Aβ concentration of 1–15 nM in the retina, up to 150 nM MRZ-99030 could be required at the protein target. In line with this consideration, MRZ-99030 was able to prevent Aβ-induced toxicity on PC12 cells, retinal ganglion cells and retinal pigment epithelium cells when present at a 10–20 fold stoichiometric excess over Aβ. Moreover, *in vivo* studies demonstrate the neuroprotective potential of MRZ-99030 after systemic and topical administration in animal models of Alzheimer's disease and glaucoma/AMD respectively.

1. Introduction

1.1. Pathophysiology of Alzheimer's disease

The pathophysiology of Alzheimer's disease (AD) is characterized by chronic, progressive neurodegeneration. The precise aetiology of AD is still not fully clarified, but is known to be complex and multifactorial, with a notable overlap between familial and non-familial forms but also with different forms of dementia such as vascular dementia. The neurodegeneration seen in AD involves early synaptotoxicity and loss of neuropil, neurotransmitter disturbances, accumulation of extracellular β-amyloid (Aβ) deposits (amyloid/senile plaques) and intracellular neurofibrils (neurofibrillary tangles, NFTs), gliosis and only at later stages overt loss of neurons and associated brain atrophy (Bell and Claudio Cuello, 2006; Citron, 2010; Heininger, 1999; Yankner, 1996). At early stages of the disease, the entorhinal cortex and hippocampus are particularly affected and this is associated with deficits in cognition/memory (Braak et al., 1993). Over the course of AD, up to 80% of neurons in the hippocampus die, and the progressive symptoms of

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AD manifest themselves as cognitive disturbances, reduced ability to cope with everyday life, and worsening of clinical global impression score (Morris, 1986).

1.2. β-amyloid

As described by Alois Alzheimer himself (Alzheimer, 1907), one of the key histopathological hallmarks of the AD brain is the presence of extracellular 'amyloid/senile plaques' around neurons and glia. Such amyloid plaques are insoluble, quasi-crystalline deposits (Lesne et al., 2006), the main component of which is Aβ – a peptide (most commonly 40–42 amino acids in length) that is formed by enzymatic cleavage of the transmembrane amyloid precursor protein (APP) (Citron, 2010; Hardy and Higgins, 1992). Due to its neurotoxic effects and accumulation in AD, Aβ is believed to be a crucial pathogenic factor in disease development, both in familial and non-familial forms. Aβ is produced by the enzymatic cleavage of APP by β-secretase (extracellular cleavage) and γ-secretase (cuts in the middle of the membrane) whereas cleavage by α-secretase precludes formation of Aβ. The 42 amino acid form, $Aβ_{1–42}$, has a higher tendency to aggregate than $Aβ_{1–40}$ and has been ascribed to be the main pathogenic form of this peptide (Citron, 2010). Aβ is continually released from neurons and glial cells into the extracellular environment where, at low concentrations and possibly in monomeric form it may play a physiological role (Puzzo et al., 2008).

1.3. Soluble β-amyloid oligomers

More recent evidence indicates that soluble oligomeric forms of Aβ, rather than the insoluble deposits, are primarily responsible for both the neurodegeneration and especially the impairment of synaptic function in AD (Barghorn et al., 2005; Demuro et al., 2010; Ferreira et al., 2011; Ferreira and Klein, 2011; Ferreira et al., 2007; Lacor et al., 2007; Wilcox et al., 2011; Xia, 2010). For example, $Aβ_{1–42}$ and Aβ oligomers were reported to be dramatically increased in the soluble fraction of Alzheimer's disease brain extracts, with oligomer levels 20-fold higher in aqueous compared with detergent extracts. Multiple oligomeric forms, including small oligomers, 56-kDa and 200-kDa assemblies were proposed by the authors to contribute to synaptic dysfunction (Sokolow et al., 2011). However, contradictory findings have also been reported by others e.g. (van Helmond et al., 2010).

APP transgenic (TG) mice expressing the E693Delta mutation, which is reported to cause AD by enhanced Aβ oligomerization without fibrillization, displayed age-dependent accumulation of intraneuronal Aβ oligomers starting at 8 months but no extracellular amyloid deposits even at 24 months (Tomiyama et al., 2010). These mice indeed already showed deficits in synaptic plasticity, learning, synaptic markers, microglial activation and tau phosphorylation at 8 months indicating that they might be a useful model of Aβ oligomer-induced pathology in the absence of amyloid plaques (Tomiyama et al., 2010). Soluble Aβ associated with (Q22, Dutch) or (G22, Arctic) mutant APP peptides was approximately 100-fold more potent than wild-type Aβ in inhibiting long term potentiation (LTP) (Klyubin et al., 2004).

These soluble Aβ oligomers are thought to promote disturbances in glutamatergic neurotransmission and also increase the phosphorylation of tau (De Felice, et al., 2007). For example, chronic treatment with nanomolar concentration of Aβ oligomers was recently reported to induce N-methyl-D-aspartate (NMDA) receptor-dependent inward calcium ion (Ca^{2+}) currents, mitochondrial Ca^{2+} overload/membrane depolarization, oxidative stress and apoptotic cell death in primary dissociated and entorhinal cortex/hippocampal organotypic cultures (Alberdi et al., 2010; Bieschke et al., 2011).

Aβ oligomers are now believed to impair neuronal function and cognition, even before the appearance of overt toxicity (Lesne et al., 2006). However, the exact pathogenic role of deposits vs. soluble forms and, in the latter case especially the major oligomeric species of Aβ involved (e.g. dimer, trimer or dodecamer), is still controversial (Bao et al., 2011; Barghorn et al., 2005; Selkoe, 2008). In contrast to soluble oligomeric forms of Aβ, this peptide in its soluble monomeric form has recently even been ascribed a physiological function and can enhance LTP at low pM concentrations (Puzzo et al., 2008), increase synaptic release probability (Abramov et al., 2009) and even protect against excitotoxic insults (Giuffrida et al., 2009).

1.4. Ocular diseases

Glaucoma and age-related macular degeneration (AMD) are leading causes of progressive vision loss and blindness worldwide with the incidence and prevalence of each disease increasing substantially with age (Friedman et al., 2004a, 2004b; Klein et al., 2002). While distinctly different entities, similarities exist among the two diseases as well as with Alzheimer's disease (AD) as all three conditions have a strong age-related incidence and chronic neurodegenerative changes seen in the eyes of glaucoma and AMD patients are similar to changes characteristic of the brains of the AD patient (Friedman et al., 2004a, 2004b; Johnson et al., 2002; Klein et al., 2002).

1.5. Glaucoma

Aβ has recently been reported to be implicated in the development of retinal ganglion cell (RGC) apoptosis in glaucoma, with evidence of caspase-3-mediated abnormal APP processing (McKinnon, 2003), increased expression of Aβ in RGCs and optic nerves in experimental glaucoma (Goldblum et al., 2007; Guo et al., 2007; Kipfer-Kauer et al., 2010; McKinnon, 2003; Zhu et al., 2009) and decreased vitreous Aβ levels (consistent with retinal Aβ deposition) in patients with glaucoma (Yoneda et al., 2005). Very recent data show that Aβ is increased in the optic nerve (McKinnon, *personal communication*) and RGC layer of glaucoma patients (Von Thun und Hohenstein-Blaul et al., 2013). Small soluble oligomeric forms of Aβ are able to induce significant RGC apoptosis *in vivo* and *in vitro* (Guo et al., 2007; Tsuruma et al., 2010; Walsh et al., 2005). It has been shown that targeting different components of the Aβ formation and aggregation pathway can reduce glaucomatous RGC apoptosis *in vivo* and therefore raises the possibility of neuroprotection in glaucoma (Guo et al., 2007).

Further evidence of a link between glaucoma and AD has emerged from studies showing that patients with AD have RGC loss associated with typical glaucomatous changes, such as optic neuropathy and visual functional impairment (Blanks et al., 1996a; 1996b; Iseri et al., 2006; Parisi et al., 2001). In addition, both diseases are chronic neurodegenerative conditions with a strong age-related incidence (Johnson et al., 2002). This finding is further supported by increasing evidence of similar pathological mechanisms involving Aβ leading to RGC loss as implicated in the brain (Johnson et al., 2002).

1.6. AMD

AMD is characterized by drusen, extracellular waste deposited between the basal surface of the retinal pigment epithelium (RPE) and Bruch's membrane. Drusen are a hallmark of early, or

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