Experimental Gerontology 108 (2018) 215–225

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

5-Methoxyindole-2-carboxylic acid (MICA) suppresses Aβ-mediated pathology in *C. elegans*

Waqar Ahmad, Paul R. Ebert*

School of Biological Sciences, The University of Queensland, Brisbane 4072, Australia

ARTICLE INFO

Section Editor: T.E. Johnson

Keywords:
Alzheimer's disease
Neurodegeneration
Amyloid beta
Energy metabolism
Dihydrolipoamide dehydrogenase (*dld*)
C. elegans

ABSTRACT

Alzheimer's disease (AD) is an age-related disease characterized by loss of memory and disrupted thinking that is associated with altered energy metabolism. Variants of an important enzyme of energy metabolism, dihydrolipoamide dehydrogenase (*dld)*, have been genetically linked to late-onset AD. Moreover, reduced activity of DLD-containing enzyme complexes is associated with AD progression. To understand how energy metabolism influences AD progression, we exposed *C. elegans* expressing human Aβ peptide to the chemical inhibitor of DLD, 2-methoxyindole-5-carboxylic acid (MICA). Expression of human Aβ in *C. elegans* causes a variety of pathologies that can be used to monitor the efficacy of treatments against proteotixicity. We found that MICA alleviated the Aβ-induced paralysis and improved cholinergic neurotransmission in *C. elegans* that express Aβ in muscle cells. MICA also reduced both hypersensitivity to serotonin and perturbation of chemotaxis associated with neuronal expression of human Aβ. Furthermore, low doses of MICA helped to alleviate an Aβ-mediated decrease in fecundity. Protection against AD pathogenesis by MICA in the *C. elegans* model was associated with a decrease in Aβ oligomerization that could be reversed by the calcium ionophore, A23187. MICA also caused a decrease in oxidative stress, which could also contribute to the protective effect of MICA against Aβ toxicity.

1. Introduction

Despite 100 years of research into Alzheimer's disease (AD), the mechanism of disease initiation and progression is poorly understood. Overproduction and deposition of extracellular amyloid beta (Aβ) plaques is a key indicator of disease progression that is associated with cognitive impairment, neuronal loss and dementia (Castellani and Perry, 2014; Hyman et al., 2012). In addition to the presence of Aβ plaques, reduced glucose energy metabolism and diminished activities of mitochondrial enzymes were found in brain tissue from late-stage AD patients (Mosconi, 2013; Shoffner, 1997; Vlassenko et al., 2012; Vlassenko et al., 2010). This decrease in energy metabolism could be (i) caused by disease progression as several late-stage postmortem studies found reduced energy metabolism in AD brain samples (Hooijmans et al., 2007; Hoyer, 1991; Hoyer, 2000; Hoyer, 2004; Hunt et al., 2007), or (ii) a cellular response designed to limit damage due to Aβ-toxicity. Recent studies on post mortem brains at various stages of disease progression support the second interpretations as the down regulation of energy metabolism occurs too early in disease progression to be either a cause or consequence of tissue damage. Rather, the authors suggested that reduced energy metabolism is a protective measure designed to minimize neuronal activity, thereby decreasing the repair burden (Liang et al., 2012; Sun et al., 2012). Regardless of the interpretation, the evidence suggests an intimate link between AD and energy metabolism.

The limited availability of AD affected brains from various stages of disease progression makes it difficult to investigate the role of energy metabolism in the process. However, a decrease in the activity of three dihydrolipoamide dehydrogenase-containing mitochondrial enzymes of energy metabolism, pyruvate dehydrogenase (PDH), α-ketoglutarate dehydrogenase (KGDH) and branched chain α-keto acid dehydrogenase (BCKDH has been observed in AD (Gibson et al., 2012; Shi et al., 2011; Simoncini et al., 2015). PDH links glycolysis to the TCA cycle and is a major control point in the regulation of aerobic respiration. Dichloroacetate, an activator of PDH induces oxidative phosphorylation and increases neuronal sensitivity to Aβ (Newington et al., 2011). In contrast, the glucose analogue, 2-deoxy-D-glucose, inhibits both glycolysis and aerobic respiration, and reduces Aβ toxicity in mouse models as well (Vilalta and Brown, 2014; Yao et al., 2011). KDGHC is the rate-limiting step of the TCA cycle (Shoffner, 1997), and may have a similar role in suppressing the rate of aerobic respiration to reduce AD pathogenesis.

There is a variety of genetic evidence that links dihydrolipoamide dehydrogenase (DLD) to AD, including genetic variants associated with

* Corresponding author.
E-mail address: p.ebert@uq.edu.au (P.R. Ebert).

https://doi.org/10.1016/j.exger.2018.04.021
Received 22 August 2017; Received in revised form 2 April 2018; Accepted 26 April 2018
Available online 27 April 2018
0531-5565/

the disease in an Ashkenazi Jewish population (Brown et al., 2007). This supports the evidence from changes in the activity of DLD-containing enzyme complexes mentioned above. In the current study we use a chemical inhibitor of DLD enzyme activity known as 5-methoxyindole-2-carboxylic acid (MICA) to determine whether it also can alleviate Aβ toxicity in *C. elegans* that express human Aβ.

MICA is a hypoglycemic agent that has been suggested as a possible drug to treat type 2 diabetes (T2DM) (Bauman and Hill, 1968; Bauman and Pease, 1969; Gold, 1974; Schillinger and Loge, 1976; Ueki et al., 1985). Evidence indicates that the metabolic disease diabetes increases the risk of AD (Nguyen et al., 2014; Sebastiao et al., 2014; Verdile et al., 2015). As both AD and diabetes share many pathological features (Ahmad, 2013; Rosales-Corral et al., 2015), it is possible that drugs used to lower hyperglycemia such as MICA might be effective in AD treatment. Although the exact mechanism whereby MICA alleviates hyperglycemia is not fully understood, MICA modifies cellular glucose levels by inhibiting glucose metabolism rather than insulin sensitivity (Arneric et al., 1984; Bauman and Pease, 1969; Hanson et al., 1969). Consistent with its action as an inhibitor of DLD, MICA inhibits the oxidation of the keto acid substrates of the enzyme complexes that contain DLD. This was reflected in reduced DLD enzyme activity in mitochondria of mice/rats treated with MICA (Bauman and Hill, 1968; Haramaki et al., 1997). Inhibition of DLD by MICA was reversible by increasing intracellular calcium levels using the calcium ionophore A23187 (CaI) (Mitra and Shivaji, 2004; Nichols and Denton, 1995).

There is no evidence regarding the role of MICA on neurodegeneration, though one possible mechanism by which MICA could influence neurodegeneration is by modifying energy metabolism. Our results showed that MICA protects against Aβ-mediated toxicity in *C. elegans* that express human Aβ. The protective effect of MICA is associated with a decrease in Aβ oligomerisation. In our study, the protective effects of MICA can be reversed using MICA inhibitor calcium ionophore A23187 (CaI).

2. Materials and methods

2.1. Nematode strains

C. elegans strains used in this study are: wild type strain N2 (Bristol) long-lived, stress resistant *dld-1* mutant, *dld-1*(*wr4*) (Cheng et al., 2003; Schlipalius et al., 2012), and the transgenic strains that express the human β-amyloid peptide in muscle cells. CL2006 (*dvIs2* [pCL12(*unc-54*/hu-Aβ42) + *pRF4*]), which produces the human $A\beta_{42}$ peptide constitutively and CL4176 (*smg*-1^{ts} [*myo*-3/Aβ42 long 3′-UTR]), in which $A\beta_{42}$ peptide expression is increased when the temperature is increased from 16 °C to 23 °C. The use of these strains as a worm model of AD was documented previously (Link, 1995). We also used strain CL2355 (*dvIs50* [pCL45 (*snb-1*::Abeta 1–42::3′ UTR(long) + *mtl-2::gfp*] I), in which Aβ is also expressed under temperature control, though in this case the peptide is expressed pan-neuronally. The control strain for CL2355 was CL2122 (*dvIs15*(*mtl-2::gfp*) (Link, 1995; McColl et al., 2009; McColl et al., 2012; Wu et al., 2006). All the strains were obtained from the Caenorhabditis Genetics Center (CGC).

2.2. Culture conditions

Worms were maintained on nematode growth medium (NGM) seeded with *E. coli* OP50 at 20 °C, except strains CL4176 and CL2355, which were maintained at 16 °C to suppress Aβ expression. Synchronized cultures for bioassays were obtained using the standard bleaching procedure (Stiernagle, 2006). Briefly, gravid hermaphrodites were exposed to a freshly prepared alkaline bleach solution (0.75 N NaOH + 1.5 N NaOCl). The worms were incubated in the bleach solution for 5 min at room temperature followed by centrifugation at 1100 RPM for 1 min at room temperature. The supernatant was then discarded, and the pelleted eggs were resuspended in M9 buffer (6 g/L Na_2HPO_4; 3 g/L KH_2PO_4; 5 g/L NaCl; 0.25 g/L $MgSO_4$ •$7H_2O$. Washing with M9 buffer was repeated 3 more times, after which the eggs were suspended in M9 buffer and allowed to hatch overnight on an orbital shaker. The resulting L1 larvae were shifted to the fresh NGM agar plates seeded with *E. coli* to initiate growth. Aβ inducible transgenic worms were initially cultivated at 16 °C for 36 h after which the temperature was increased to 23 °C for 36 h except for the paralysis assay for which the temperature was further increased to 25 °C to maximize the effect of the Aβ transgene. Aβ constitutive expressing worms were cultured at 20 °C Phenotypes of the worms were monitored by visual observation under a microscope or quantified using the WormScan procedure (Mathew et al., 2012).

2.3. Gene suppression by RNAi

The *E. coli* strain SJJ_LLC1.3 (Source Bioscience), which expresses double-stranded RNA of the *dld-1* gene, was fed to the worms to trigger *dld-1* gene suppression (Kamath and Ahringer, 2003). Briefly, the bacteria were cultured in LB medium containing 100 μg/mL ampicillin overnight with shaking at 37 °C. 300 μL of this bacterial culture was transferred to NGM plates containing 100 μg/mL ampicillin and 1 mM IPTG. The plates were incubated at 25 °C overnight to allow the bacteria to grow. Synchronized L1 worms were transferred to the bacterial plates and kept at 16 °C for 36 h. After a further 36 h at 20 or 25 °C, the worms were ready for use in gene expression, protein abundance and worm activity assays as described below. Mock gene suppression controls were treated in exactly the same way except that the worms were fed bacterial strain (HT115), which contained the plasmid vector without any specific gene fragment corresponding to a *C. elegans* gene.

2.4. Paralysis assay

Synchronized L1 stage CL4176 worms were transferred to NGM plates. To assess the role of chemical inhibition on paralysis behaviour of Aβ transgenic strains, different concentrations of chemicals were used in normal NGM plates. Transgenic worms were exposed to these chemicals continuously - before, during and after the induction of Aβ expression. After 36 h at 16 °C, worms were shifted to 25 °C and scored for paralysis every 24 h. Scoring began 24 h after the temperature was raised to increase transgene expression.

2.5. Aldicarb and levamisole assays

Synchronized L1 worms were placed at 20 °C on NGM plates or NGM plates supplemented with 5 mM MICA that had been seeded with a lawn of OP50 *E. coli.* The worms were allowed to grow until they reached the L4 stage of development, at which time they were shifted to new NGM plates containing 1 mM aldicarb, an ACh sterase inhibitor (Mahoney et al., 2006) or 0.2 mM levamisole, a cholinergic agonist (Lewis et al., 1987). Worms that had matured in the absence of MICA were exposed to the toxin without MICA and those exposed to MICA during maturation were, likewise, exposed to MICA during exposure to the toxin. The number of active worms was counted every half an hour until all worms became paralysed.

2.6. Serotonin assay

To determine the level of Aβ-induced serotonin hypersensitivity, serotonin (creatinine sulfate salt) was first dissolved in M9 buffer to 1 mM as described previously (Dosanjh et al., 2010; Zhang et al., 2005). Synchronized worms were then washed with M9 buffer, and worms were transferred to 200 μL of the 1 mM serotonin solution in 12-well assay plates. The worms were scored as either active or paralysed after 5 min.