Mechanisms of Ageing and Development 133 (2012) 267–274

Contents lists available at SciVerse ScienceDirect

Mechanisms of Ageing and Development

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

Neuroprotection by the multitarget iron chelator M30 on age-related alterations in mice

Lana Kupershmidt, Tamar Amit, Orit Bar-Am, Moussa B.H. Youdim, Orly Weinreb *

Eve Topf Centers of Excellence for Neurodegenerative Diseases Research and Department of Pharmacology, Rappaport Family Research Institute Technion-Faculty of Medicine, Haifa 31096, Israel

ARTICLE INFO

Article history:
Received 7 December 2011
Received in revised form 13 February 2012
Accepted 1 March 2012
Available online 10 March 2012

Keywords:
Ageing
Iron chelator
Monoamine oxidase inhibitor
Neuroprotection
β-Amyloid plaques

ABSTRACT

Based on a multimodal drug design paradigm, we have synthesized a multifunctional non-toxic, brain permeable iron chelating compound, M30, possessing the neuroprotective N-propargyl moiety of the anti-Parkinsonian drug, monoamine oxidase (MAO)-B inhibitor, rasagiline and the antioxidant-iron chelator moiety of an 8-hydroxyquinoline derivative of the iron chelator, VK28. Here, we report that a chronic systemic treatment of aged mice with M30 (1 and 5 mg/kg; 4 times weekly for 6 months), had a significant positive impact on neuropsychiatry functions and cognitive age-related impairment. M30 significantly reduced cerebral iron accumulation as demonstrated by Perl's staining, accompanied by a marked decrease in cerebral β-amyloid plaques. In addition, our results demonstrate that M30 caused a significant inhibition of both MAO-A and -B activities in the cerebellum of aged mice, compared with vehicle-treated aged control mice. In summary, the present study indicates that the novel MAO inhibitor/iron chelating drug, M30, acting against multiple brain targets could reverse age-associated memory impairment and provide a potential treatment against the progression of neurodegeneration in ageing.

1. Introduction

Brain ageing is associated with the progressive imbalance between antioxidant defenses and intracellular generation of reactive oxygen species (ROS). The age-related increase in oxidative brain damage has been mainly exemplified by lipid peroxidation, protein oxidation and oxidative modifications in DNA (Droge and Schipper, 2007). Previously, age-related memory impairment has been shown to be associated with a decrease in brain and plasma antioxidants (Berr et al., 2000; Perkins et al., 1999; Rinaldi et al., 2003).

It is also suggested that iron accumulation in the brain is capable of initiating free-radicals reactions, which subsequently induce progressive loss of neurons, followed by a decrement in neuronal function characteristic of the ageing process (Sayre et al., 2000; Zecca et al., 2004). Indeed, it has become apparent that iron progressively accumulates in the brain as a function of age, and that iron-induced oxidative stress (OS) can cause neurodegeneration (Zecca et al., 2004). This process is quite specific and involves the accumulation of iron-containing molecules in specific brain regions (such as the striatum, substantia nigra (SN) and cerebellum (Bartzokis et al., 1994; Benkovic and Connor, 1993) that are preferentially targeted in neurodegenerative diseases (e.g. Parkinson's disease (PD) and Alzheimer's disease (AD), multiple system atrophy and Huntington's disease (Bartzokis et al., 1999; Floor, 2000; Vymazal et al., 1999; Yokel, 2006). In preclinical studies, it was found that iron accumulation altered antioxidant capacity and thiol redox state in aged animals (Suh et al., 2005). It thus may be speculated that regulated suppression of labile iron levels would decrease the rate of accumulation of over-oxidized materials inside the cells and in this way favorably influence the ageing process. An additional prominent feature that accompanies ageing is an increase in the levels of monoamine oxidase (MAO), an enzyme that catalyzes the oxidative deamination of neurotransmitters, in which the by-product H_2O_2 is subsequently generated (Kumar and Andersen, 2004). Several studies have also reported high levels of MAO in the brain in neurodegenerative diseases, such as PD and AD (Bortolato et al., 2008; Riederer et al., 2004).

We have recently developed the multifunctional neuroprotective compound, M30 with dual iron chelating and MAO-A and B inhibitory activity that combines the antioxidant chelator moiety of an 8-hydroxyquinoline derivative of the brain permeable iron

Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; APP, amyloid precursor protein; DFO, defferoxamine; DA, dopamine; MAO, monoamine oxidase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; OS, Oxidative stress; PD, Parkinson's disease; PSEN, presenilin; ROS, reactive oxygen species; SOD, superoxide dismutase; SN, sabstantia nigra; TH, tyrosine hydroxylase.

* Corresponding author at: Department of Pharmacology, Technion-Faculty of Medicine, P.O.B. 9697, 31096 Haifa, Israel. Tel.: +972 4 8295290; fax: +972 4 8513145.
E-mail address: worly@tx.technion.ac.il (O. Weinreb).

0047-6374/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mad.2012.03.001

chelator VK28 and the propargyl moiety of the anti-Parkinsonian MAO-B inhibitor, rasagiline (Azilect®, Teva, Pharmaceutical Inc.) (Youdim and Buccafusco, 2005; Zheng et al., 2005a,b). M30 was found to possess a wide range of pharmacological activities, including neurorescue effects, induction of neuronal differentiation and regulatory action on the Alzheimer's amyloid precursor protein (APP) and amyloidogenic Aβ peptides (Avramovich-Tirosh et al., 2007a,b). In in vivo studies, M30 was shown to prevent the loss of mouse tyrosine hydroxylase (TH)-positive neurons; iron increase; and microglial activation in the ipsilateral SN-induced by post-intranigral injection of the ubiquitin-proteasome inhibitor, lactacystin (Zhu et al., 2007). Moreover, M30 prevented the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced striatal dopamine (DA) depletion, as well as restored nigrostriaral DA neurons in a post-MPTP mouse model of PD (Gal et al., 2005, 2010). In C57BL mice, M30 treatment was found to significantly up-regulate a number of neuroprotective–adaptive mechanisms and pro-survival signaling pathways in the brain (Kupershmidt et al., 2011). Recently, we have shown that M30 improved cognitive impairment and reduced Alzheimer's-like neuropathology in a mouse model of AD (Kupershmidt et al., 2012).

In this study, we examined the possible effects of the novel multifunctional compound, M30 on improving cognitive behavior in ageing mice. Additionally, the regulatory effects of M30 on cerebral MAO activity and amyloid deposition in aged mice were evaluated.

2. Materials and methods

2.1. Materials

Mouse monoclonal anti-Aβ (17-24) antibody (4G8) was purchased from Covance (Princeton, NJ, USA). Donkey anti-rabbit IgG fluorescein-conjugated antibody was from Jackson Immuno-Research laboratories Inc. (Baltimore, MD, USA). Mounting medium was from Vector Laboratories (Peterborough, UK). Other chemicals and reagents were of the highest analytical grade and purchased from local commercial sources. The multifunctional iron chelator M30 (5-[N-methyl-N-propargylaminomethyl] -8-hydroxyquinoline) (MW 299.3) was synthesized (Gal et al., 2005) and kindly provided by Varinel Inc. (Philadelphia, PA USA).

2.2. Animal treatment

All procedures were carried out in accordance with the National Institutes of Health Guide for care and Use of Laboratory Animals, and were approved by the Animal Ethics Committee of the Technion, Haifa, Israel. Control young (2 month-old) and aged (15 month-old) male C57BL mice were obtained from Harlan Laboratories, Inc (Israel). M30 (1 and 5 mg/kg) and vehicle were administered to aged mice (10 mice per each experimental group) by the oral gavage method, as recommended (Ludolph et al., 2007), 4 times a week for 6 months. The effective doses of M30 used in all experiments were chosen, based on our recent neuroprotective animal studies in the MPTP and lactacystin mice models of neurodegeneration (Gal et al., 2005; Zhu et al., 2007), G93A SOD1 mice model of amyotrophic lateral sclerosis (ALS) (Kupershmidt et al., 2009) and the APP/presenilin (PSEN)1 model of AD (Kupershmidt et al., 2012). Mice were weighed once a week. 4 weeks before the end of drug treatment, behavioral tests (object recognition, nesting behavior, SHIRPA, and open field performance) were performed, as described below, to assess the effects of the drug on cognitive and non-cognitive behavior.

2.3. Behavioral analysis

2.3.1. Object recognition test

Mice were trained and tested in the novel object recognition task, as previously described (de Lima et al., 2008). Training in the object recognition task took place in the same arena used for the open field. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test required that the mice recalled which of the two plastic objects they had been previously familiarized with. Twenty-four hours after arena exploration, training was conducted by placing individual mice into the field, in which two identical objects (objects A1 and A2) were positioned in two adjacent corners, 9 cm from the walls. Animals were left to explore the objects until they had accumulated 30 s of total object exploration time, or for a maximum of 20 min. In a short-term memory test, given 1.5 h after training, the mice explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects presented similar textures, colors, and sizes, but distinctive shapes. A recognition index was calculated for each animal and expressed by the ratio TB/(TA + TB) [TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B]. In a long-term memory test, given 24 h after training, the same mice explored the field for 5 min in the presence of a familiar object A and a novel object C. Recognition memory was evaluated as for the short-term memory test. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration, as described by Simola et al. (2008).

2.3.2. Open field performance

The open field was a 40 cm × 45 cm arena surrounded by 50 cm high walls. The floor of the arena was divided into 12 equal squares by black lines. Animals were placed in the near left corner and left to explore the field freely for 5 min (de Lima et al., 2005). Latency to start locomotion, line crossing, rearing and the number of fecal pellets produced were counted (de Lima et al., 2005).

2.3.3. Nest building assay

Mice were housed in single cages containing sawdust for 3 days. On the first day of testing, two pieces of cotton (5 cm × 5 cm, Nestlets; Ancare, Bellmore, NY, USA) were introduced in the home cage to permit nesting. The presence and quality of nesting were rated 1 day later on a 5-point scale ranging from 1 to 5 (Deacon et al., 2008; Filali et al., 2009) as follows: 1 = nestlet not noticeably touched, 2 = nestlet partially torn up, 3 = mostly shredded, but often with no identifiable nest site, 4 = an identifiable, but flat nest, and 5 = a near perfect nest.

2.3.4. Modified SHIRPA protocol

Modified SHIRPA, a behavioral and functional analysis of mouse phenotype, was performed as recommended by MRC Harwell, an international centre for mouse genetics (http://empress.har.mrc.ac.uk/browser/?sop_id=10_002_0). The evaluation scores were:

Body position: 0 = inactive; 1 = active; 2 = excessive activity; Tremor: 0 = absent; 1 = present; Palpebral Closure: 0 = eyes open; 1 = eyes closed; Coat Appearance: 0 = tidy and well groomed coat; 1 = irregularities such as piloerection; Whiskers: 0 = present; 1 = absent; Lacrimation: 0 = absent; 1 = present; Defecation: 0 = present; 1 = absent; Tansfer Arousal: 0 = extended freeze (over 5 s); 1 = brief freeze followed by movement; 2 = immediate movement; Gait: 0 = fluid movement and approximately 3 mm pelvic elevation; 1 = lack of fluidity in movement; Tail Elevation: 0 = dragging; 1 = horizontal extension; 2 = elevated/straub tail; Startle Response: 0 = none; 1 = Preyer reflex (backwards flick of the pinnae); 2 = reaction in addition to the Preyer reflex; Touch Escape: 0 = no response; 1 = response to touch; 2 = flees prior to touch; Positional passivity: 0 = struggles when held by the tail; 1 = struggles when held by the neck (loose scruff between the forefinger and thumb); 2 = struggles when laid supine; 3 = no struggle; Skin Color: 0 = blanched; 1 = pink; 2 = bright, deep red flush;

Trunk Curl: 0 = absent; 1 = present; Limb Grasping: 0 = absent; 1 = present; Pinna Reflex: 0 = present; 1 = absent; Corneal Reflex: 0 = present; 1 = absent; Contact Righting Reflex: 0 = present; 1 = absent; Evidence of Biting: 0 = none; 1 = biting in response to handling; Vocalisation: 0 = none; 1 = vocal. Three trials were given to each animal and the best performance determined the coded score at each task.

2.4. Preparation of tissue slides, immunohistochemistry studies and Perl's staining

Brain hemispheres were post-fixed in 4% (vol/0.1 M PBS vol) paraformaldehyde (48 h, 4 °C) and cryoprotected by 30% sucrose (48 h, 4 °C). Six series of 40-μm coronal sections were collected in PBS on a freezing–sliding microtome. Immunohistochemistry for Aβ was performed on series 1 and series 2 and 3 were used for Thioflavin S and Perl's staining, Thioflavin S staining for fibrillar plaques was performed by incubating slides in 1% aques solution of Thioflavin S for 10 min, followed by rinsing in 80 and 95% ethanol and then distilled water. For immunehistochemistry studies, staining slides were incubated in PBS containing 10% donkey serum at 37 °C for 1 h to block nonspecific staining. Slides were incubated at 4 °C overnight with a mouse monoclonal anti-Aβ (17–24) antibody (4G8), diluted in PBS containing 1% donkey serum and 0.05% Triton X-100 (1:200). Slides were rinsed twice with PBS for 10 min before addition of donkey secondary IgG–Fluorescein-conjugated antibody diluted in PBS, 1% donkey serum and 0.05% Triton X-100. After incubation at RT in the dark for 1 h, slides were rinsed 3 times in PBS and coverslips were mounted with Vectashield containing 4′-6-diamidino-2-phenylindole (DAPI) for nuclear staining. For Per's staining, slides were rinsed in 0.1 M PBS followed by a 10 min incubation in 0.1% Triton-100/PBS solution. Next, slides were immersed in a solution containing equal amounts of 4% potassium ferrocyanide and 4% hydrochloric acid for 30 min in the dark. After PBS rinses and 10 min incubation in 0.1% Triton-100/PBS, iron was visualized using 3,3-diaminobenzidine (DAB; Vector) for 30 min at RT in the dark. Sections were rinsed in distilled water, quickly dehydrated and coverslipped. Immunofluorescence and iron labeling were observed using a Bio Rad Radiance 2000 confocal system, supported with Laser-Sharp 2000 software. No fluorescence is expected to be detected when the primary antibody is omitted. The proportions of the lesion occupied by iron and Thioflavin S and Aβ positive plaques were performed using Laser-Sharp 2000 software. Eight images of slides were obtained per each region of