Mol Neurobiol (2012) 46:217–220
DOI 10.1007/s12035-012-8304-7

Multi-target, Neuroprotective and Neurorestorative M30 Improves Cognitive Impairment and Reduces Alzheimer's-Like Neuropathology and Age-Related Alterations in Mice

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

Received: 7 July 2012 / Accepted: 10 July 2012 / Published online: 31 July 2012

Abstract Based on a multimodal drug design strategy for age-related neurodegenerative diseases, we have synthesized a multifunctional nontoxic, brain-permeable iron-chelating compound, M30, possessing the neuroprotective *N*-propargyl moiety of the anti-Parkinsonian drug, monoamine oxidase-B inhibitor, rasagiline and the antioxidant–iron chelator moiety of the 8-hydroxyquinoline derivative of the iron chelator, VK28. In the present short overview, we describe the neuroprotective and the neurorestorative activity of M30, acting against multiple brain targets, including regulation on amyloid β, neurogenesis, and activation of hypoxia inducible factor signaling pathways. The diverse pharmacological properties and several pathological targets of M30 make this drug potential valuable for therapeutic strategy of Alzheimer's-like neuropathology and aging.

Keywords Alzheimer's disease · Ageing · Iron chelator · Multi-functional drug · Neuroprotection · β amyloid plaques · Hypoxia inducible factor

Introduction and Discussion

Increasing evidence has indicated that in ageing and Alzheimer's disease (AD) there is dysregulation of brain iron, which contributes to the process of oxidative stress and regulation and processing of amyloid precursor protein (APP) resulting in generation of Aβ peptide, with formation of toxic Aβ oligomers [2–5]. Based on a multimodal drug design paradigm, we have designed and synthesized a series of multifunctional (multi-target) non-toxic, brain-permeable iron-chelating compounds for AD. The multi-target M30 [6, 7] (Fig. 1) possesses the neuroprotective N-propargyl moiety of our anti-Parkinsonian drug, monoamine oxidase (MAO)-B inhibitor, rasagiline (Azilect) and the antioxidant–iron chelator moiety of an 8-hydroxyquinoline derivative of our iron chelator, VK28 [8]. Previously, we have shown that M30 is a potent brain selective monoamine oxidase A and B inhibitor, with little inhibition of liver and small intestine [9] and thus, has limited potentiation of tyramine sympathomimetic activity ("The Cheese Reaction")

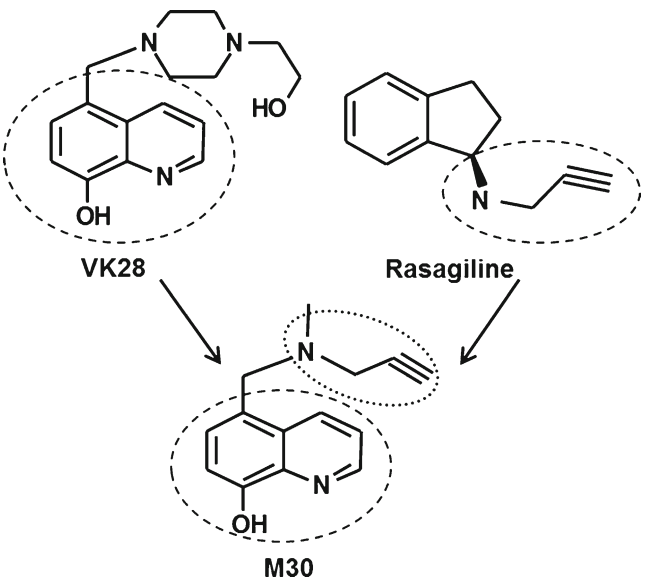

Fig. 1 Chemical structure of the brain-permeable multimodal hybrid iron-chelating drug, M30 (5-[N-methyl-N-propargylaminomethyl]-8-hydroxyquinoline), containing the propargyl moiety of the anti-Parkinsonian MAO-B inhibitor drugs, rasagiline and the antioxidant–iron-chelating moiety of VK28 (5-[4-(2-hydroxyethyl) piperazine-1-ylmethyl]-quinoline-8-ol) [6]

L. Kupershmidt · T. Amit · O. Bar-Am · O. Weinreb · M. B. H. Youdim (✉)
Eve Topf and USA National Parkinson Foundation Centers of Excellence for Neurodegenerative Diseases Research and Department of Pharmacology, Technion-Faculty of Medicine, P.O. Box 9697, Haifa 31096, Israel
e-mail: youdim@tx.technion.ac.il

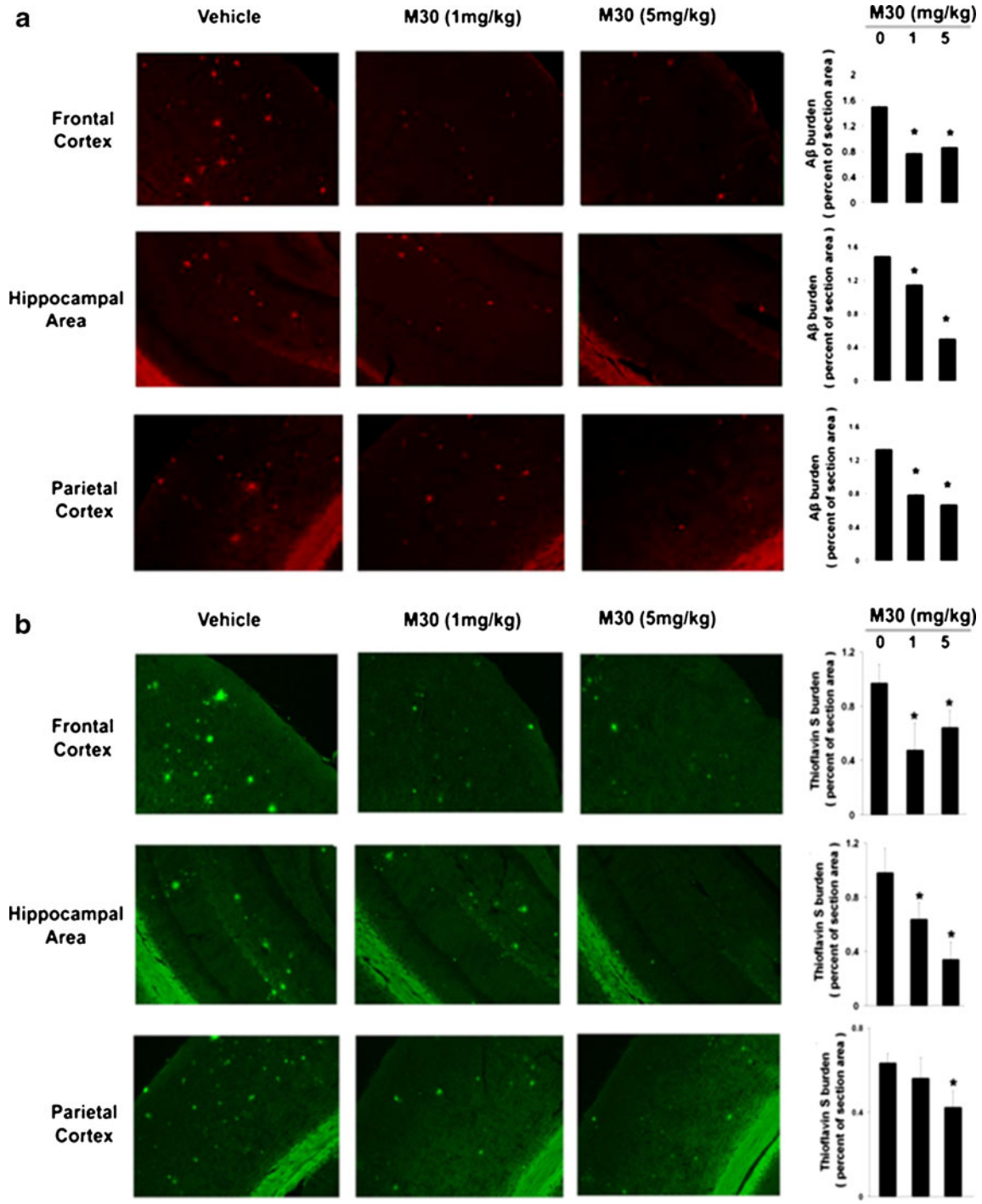

Fig. 2 Effect of M30 treatment on cerebral amyloidosis in APP/PS1 mice. **a** Immunofluorescence images of mouse brain sections from the indicated regions stained with Thioflavin S. Percentages of Thioflavin S-positive burden were calculated by quantitative image analysis [21]. **b** Immunohistochemical images showing brain coronal frozen sections from the frontal cortex, hippocampus and parietal cortex stained with Aβ (6E10) antibody. Percentages of Aβ antibody-immunoreactive Aβ plaques were calculated by quantitative image analysis. Data are mean ± SEM ($n=3$ animals per group; four to eight separate fields for each animal). *$p<0.05$ vs APP/PS1 vehicle-treated mice [21]

[10]. This "cheese effect" is one of the limiting factors of non-selective MAO inhibitors in the clinic.

In two models of Parkinson's disease, namely N-methyl-4-phenyl-tetrahydropyridine (MPTP) and lactacystin, an inhibitor of ubiquitin–proteasome system, M30 has neuroprotective and neurorestorative activity in post-degeneration of nigrostriatal dopamine neurons [9, 11, 12]. This has been attributed to neurogenesis as seen by hippocampal BrdU

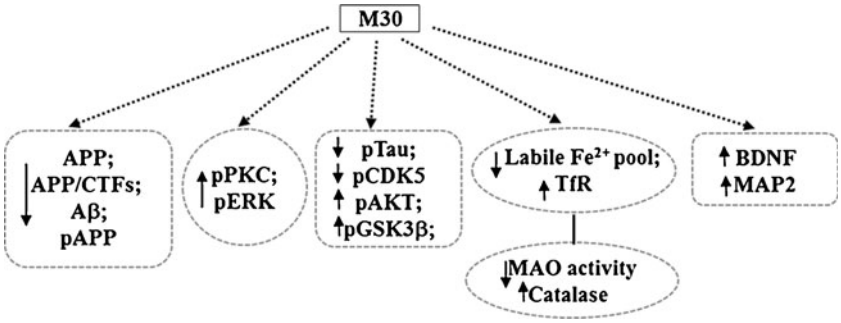

Fig. 3 Schematic diagram illustrating the potential targets identified for the multi-target iron-chelating compound, M30, as potential therapeutic for AD [20, 21]

deposit observed in the MPTP-treated animal [11]. The neurogenesis could be related to the ability of M30 to induce activation of hypoxia inducible factor (HIF-1α), via inhibation of the activity of iron-dependent prolyl-4-hydroxylase that regulates HIF-1α [13]. Activation of prolyl-4-hydroxylase results in degradation of HIF-1 by the ubiquitin–proteasome system, while its inhibition results in stabilization/activation of HIF-1α and the consequent activation of a brood set of HIF-1 target genes [14–16]. HIF-1 is known to regulate numerous antioxidant genes and a battery of neurotrophins including brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor, erythropoietin and enolase [17, 18, 19]. Indeed, we have shown that M30 does induce expression level of the neurotrophins BDNF and glial cell-derived neurotrophic factor in neuronal cell cultures and in mice brain [18, 19]. We have recently reported [20] that a chronic systemic treatment of aged mice with M30 (1 and 5 mg/kg; four 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 Aβ [20]. In addition, our results demonstrate that M30 caused a significant irreversible inhibition of both MAO-A and MAO-B activities in the cerebellum of aged mice, compared with vehicle-treated aged control mice. These studies in aged mice indicate that the novel MAO inhibitor/iron-chelating drug, M30, acting against multiple brain targets reverses age-associated memory impairment and thus may provide a potential treatment against the progression of neurodegeneration in ageing. We have further extended these studies in APP/presenilin 1 (PS1) double transgenic (Tg) mouse model of AD [21]. To evaluate the therapeutic effect of M30 on AD neuropathology and deficits of spatial learning and memory in the double-Tg AD mice [21].

Systematic treatment of APP/PS1 Tg mice with M30 (1 and 5 mg/kg, PO) for 9 months, highly significantly attenuated cognitive impairments in a variety of tasks of spatial learning and memory retention, working memory, learning abilities, anxiety levels and memory for novel food and nesting behaviour [21]. Furthermore, we found that M30 reduced cerebral (hippocampus) iron accumulation. This was accompanied by a marked decrease in several AD-like phenotypes, including cerebral (hippocampus, frontal cortex and parietal cortex) APP levels, Aβ levels and plaques (Fig. 2), phospho-APP and phospho-tau. Signalling studies revealed that M30 markedly downregulated the levels of phosphorylated cyclin-dependent kinase 5 and increased protein kinase B and glycogen synthase kinase 3β phosphorylation (Fig. 3).

Accumulation and deposition of brain iron is central to various neuropathological processes in AD, including oxidative stress, amyloid deposition and tau phosphorylation [21]. Thus, the concept of iron chelation and inhibition of MAO A and B, resulting in prevention of generation of reactive hydroxyl radical from hydrogen peroxide liberated by MAO reaction, holds considerable promise as a therapeutic strategy for AD pathogenesis. Here, for the first time, we have demonstrated that when systemically administered to APP/PS1 mice, our novel multifunctional iron chelator/radical scavenger compound, M30, effectively reduced Aβ accumulation and tau phosphorylation and attenuated memory deficits. These findings suggest that M30 is a potential therapeutic agent for the prevention and treatment of AD. Furthermore, M30 being a potent irreversible MAO A and B possesses anti-Parkinsonian and anti-depressant activities [9]. These properties could be important for the treatment of Parkinsonism seen in 40 % of AD subject (Lewy Body disease) and predisposition to depression in AD subjects, where there are deficit in raphe nucleus (serotonin) and locus coeruleus (noradrenaline).

Commercial interest MBH Youdim is the scientific founder of Varinel Inc and has commercial interest in M30 drug.

References

1. Weinreb O, Amit T, Mandel S, Kupershmidt L, Youdim MB (2010) Neuroprotective multifunctional iron chelators: from redox-sensitive process to novel therapeutic opportunities. Antioxid Redox Signal 13(6):919–949
2. Bandyopadhyay S, Huang X, Lahiri DK, Rogers JT (2010) Novel drug targets based on metallobiology of Alzheimer's disease. Expert Opin Ther Targets 14(11):1177–1197

3. Olivares D, Huang X, Branden L, Greig NH, Rogers JT (2009) Physiological and pathological role of alpha-synuclein in Parkinson's disease through iron mediated oxidative stress: the role of a putative iron-responsive element. Int J Mol Sci 10(3):1226–1260
4. Roberts BR, Ryan TM, Bush AI, Masters CL, Duce JA (2012) The role of metallobiology and amyloid-β peptides in Alzheimer's disease. J Neurochem 120(Suppl 1):149–166
5. Smith MA, Zhu X, Tabaton M, Liu G, McKeel DW Jr, Cohen ML, Wang X, Siedlak SL, Dwyer BE, Hayashi T, Nakamura M, Nunomura A, Perry G (2010) Increased iron and free radical generation in preclinical Alzheimer disease and mild cognitive impairment. J Alzheimers Dis 19(1):363–372
6. Zheng H, Weiner LM, Bar-Am O, Epsztejn S, Cabantchik ZI, Warshawsky A, Youdim MB, Fridkin M (2005) Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. Bioorg Med Chem 13(3):773–783
7. Zheng H, Fridkin M, Youdim MB (2012) From antioxidant chelators to site-activated multi-target chelators targeting hypoxia inducing factor, beta-amyloid, acetylcholinesterase and monoamine oxidase A/B. Mini Rev Med Chem 12(5):364–370
8. Shachar DB, Kahana N, Kampel V, Warshawsky A, Youdim MB (2004) Neuroprotection by a novel brain permeable iron chelator, VK-28, against 6-hydroxydopamine lesion in rats. Neuropharmacology 46 (2):254–263
9. Gal S, Zheng H, Fridkin M, Youdim MBH (2005) Novel multifunctional neuroprotective iron chelator-monoamine oxidase inhibitor drugs for neurodegenerative diseases. In vivo selective brain monoamine oxidase inhibition and prevention of MPTP-induced striatal dopamine depletion. J Neurochem 95(1):79–88
10. Gal S, Abassi ZA, Youdim MB (2010) Limited potentiation of blood pressure in response to oral tyramine by the anti-Parkinson brain selective multifunctional monoamine oxidase-AB inhibitor, M30. Neurotox Res 18(2):143–150
11. Gal S, Zheng H, Fridkin M, Youdim MB (2010) Restoration of nigrostriatal dopamine neurons in post-MPTP treatment by the novel multifunctional brain-permeable iron chelator-monoamine oxidase inhibitor drug, M30. Neurotox Res 17(1):15–27
12. Zhu W, Xie W, Pan T, Xu P, Fridkin M, Zheng H, Jankovic J, Youdim MB, Le W (2007) Prevention and restoration of lactacystin-induced nigrostriatal dopamine neuron degeneration by novel brain-permeable iron chelators. FASEBJ 21(14):3835–3844
13. Kupershmidt L, Weinreb O, Amit T, Mandel S, Carri MT, Youdim MB (2009) Neuroprotective and neuritogenic activities of novel multimodal iron-chelating drugs in motor-neuron-like NSC-34 cells and transgenic mouse model of amyotrophic lateral sclerosis. FASEB J 23(11):3766–3779
14. Smith TG, Talbot NP (2010) Prolyl hydroxylases and therapeutics. Antioxid Redox Signal 12(4):431–439
15. Gorres KL, Raines RT (2010) Prolyl 4-hydroxylase. Crit Rev Biochem Mol Biol 45(2):106–124
16. Harten SK, Ashcroft M, Maxwell PH (2010) Prolyl hydroxylase domain inhibitors: a route to HIF activation and neuroprotection. Antioxid Redox Signal 12(4):459–480
17. Chavez A, Miranda LF, Pichiule P, Chavez JC (2008) Mitochondria and hypoxia-induced gene expression mediated by hypoxia-inducible factors. Ann N Y Acad Sci 1147:312–320
18. Avramovich-Tirosh Y, Bar-Am O, Amit T, Youdim MBH Weinreb O (2010). Up-regulation of hypoxia-inducible factor (HIF) -1a and HIF-target genes in cortical neurons by the novel multifunctional iron chelator anti-Alzheimer drug, M30. Curr Alzheimer Res 7(4): 300–306
19. Kupershmidt L, Weinreb O, Amit T, Mandel S, Bar-Am O, Youdim MB (2011) Novel molecular targets of the neuroprotective/neurorescue multimodal iron chelating drug M30 in the mouse brain. Neuroscience 189:345–358
20. Kupershmidt L, Amit T, Bar-Am O, Youdim MB, Weinreb O (2012) Neuroprotection by the multitarget iron chelator M30 on age-related alterations in mice. Mech Ageing Dev 133(5):267–274
21. Kupershmidt L, Amit T, Bar Am O, Youdim MB, Weinreb O (2012) The novel multi-target iron chelating-radical scavenging compound M30 possesses beneficial effects on major hallmarks of Alzheimer's disease. Antioxid Redox Signal 17 (6):860–877