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Neuroprotective and neurorestorative activities of a novel iron chelator-brain selective monoamine oxidase-A/monoamine oxidase-B inhibitor in animal models of Parkinson's disease and aging

Orit Bar-Am [a], Tamar Amit [a], Lana Kupershmidt [a], Yuval Aluf [a], Danit Mechlovich [a], Hoda Kabha [a], Lena Danovitch [a], Vincent R. Zurawski [a], Moussa B.H. Youdim [a,b,*], Orly Weinreb [a,b]

[a] Varinel Pharmaceuticals Ltd, New Southern Industrial Park, Yokneam, Israel
[b] Eve Topf Center, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

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ABSTRACT

Recently, we have designed and synthesized a novel multipotent, brain-permeable iron-chelating drug, VAR10303 (VAR), possessing both propargyl and monoamine oxidase (MAO) inhibitory moieties. The present study was undertaken to determine the multiple pharmacological activities of VAR in neurodegenerative preclinical models. We demonstrate that VAR affords iron chelating/iron-induced lipid-peroxidation inhibitory potency and brain selective MAO-A and MAO-B inhibitory effects, with only limited tyramine-cardiovascular potentiation of blood pressure. The results show that in 6-hydroxydopamine rat (neuroprotection) and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse (neurorescue) Parkinson's disease models, VAR significantly attenuated the loss of striatal dopamine levels, markedly reduced dopamine turnover, and increased tyrosine-hydroxylase levels. Furthermore, chronic systemic treatment of aged rats with VAR improved cognitive behavior deficits and enhanced the expression levels of neurotrophic factors (e.g., brain-derived neurotrophic factor, glial cell-derived neurotrophic factor, and nerve growth factor), Bcl-2 family members and synaptic plasticity in the hippocampus. Our study indicates that the multitarget compound VAR exerted neuroprotective and neurorestorative effects in animal models of Parkinson's disease and aging, further suggesting that a drug that can regulate multiple brain targets could be an ideal treatment-strategy for age-associated neurodegenerative disorders.

1. Introduction

Accumulating evidence have demonstrated that increased monoamine oxidase (MAO)-B activity, excessive iron accumulation, and reduced antioxidant activities in the brain are essential pathogenic factors in neurodegenerative diseases (such as, Parkinson's disease [PD], Alzheimer's disease, amyotrophic lateral sclerosis, Huntington disease, and Friedreich ataxia) (Youdim and Buccafusco, 2005; Zecca et al., 2004). In these neurodegenerative disorders, as well as in the regular aging, iron accumulation has been observed in specific brain regions (Jellinger, 1999; Jellinger et al., 1993; Zecca et al., 2004). For example, studies have shown that iron concentrations are significantly elevated in parkinsonian substantia nigra pars compacta and within the melanized dopamine (DA) neurons (Gerlach et al., 2006; Gotz, 2004; Zecca et al., 2004). Similar results have also been reported in the 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal PD models (Gerlach et al., 2000; He et al., 1996; Youdim et al., 2004; Zecca et al., 2004). Evidence has shown that significant accumulation of iron in white matter tracts and nuclei throughout the brain precedes the onset of neurodegeneration and movement disorder symptoms (LaVaute et al., 2001). Indeed, it is well established that iron induces oxidative stress, because it initiates the Fenton reaction, resulting

* Corresponding author at: Eve Topf Center, Faculty of Medicine, Technion-Israel Institute of Technology, P.O.Box. 9697, 31096 Haifa, Israel. Tel.: +972 4 8295290; fax: +972 4 8513145.
E-mail address: youdim@tx.technion.ac.il (M.B.H. Youdim).

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in increased formation of hydroxyl radicals that cause damage to DNA, proteins, lipids, and ultimately cell death associated with neurodegeneration (Jellinger, 1999; Zecca et al., 2004).

Currently, simultaneous modulation of cascades of neurotoxic events, involved in the neurodegenerative process, by using a single multitarget drug is the most promising therapeutic approach for treatment of neurodegenerative disorders. The challenge of designing pluripotential and/or multitarget drugs is to combine multiple pharmacological moieties to a basic active molecule (Hopkins et al., 2006; Keith et al., 2005). We have therefore designed and synthesized a series of multitarget, nontoxic, brain permeable iron chelators with potent MAO inhibitory activity, based on chemically hybridizing the neuroprotective and MAO inhibitory moiety, N-propargyl, associated with the anti-parkinsonian/MAO-B inhibitor, rasagiline (Azilect), into the 8-hydroxyquinoline-containing pharmacophore of the prototype brain permeable iron chelator VK-28 (Zheng et al., 2005a, 2005b). Among these compounds, M30 and HLA20 were previously shown to exert iron-chelating potency, cytoprotective anti-apoptotic properties, and inhibition of iron-induced membrane lipid-peroxidation features (Zheng et al., 2005a, 2005b). In vivo studies reported that M30 showed neuroprotective effects in animal models of PD, induced either by MPTP (Gal et al., 2005, 2010a, 2010b) or the proteasome inhibitor lactacystin (Zhu et al., 2007), Alzheimer's disease (Kupershmidt et al., 2012b), amyotrophic lateral sclerosis (Kupershmidt et al., 2010), and aging (Kupershmidt et al., 2012a).

Another multifunctional member of this series is the iron chelating/MAO inhibitory compound VAR10303 (VAR). Considering possible pharmacological differences between M30 and VAR, regarding metabolic activity, pharmacodynamics and pharmacokinetics, stability and resistance mechanisms, as well as safety and tolerability, we have characterized in the present study the iron chelating and anti-oxidative properties of VAR and analyzed its in vivo neuropharmacologic actions, in terms of MAO inhibition in the brain and periphery, levels and/or turnover of striatal monoamines and pressor response to tyramine. In addition, we sought to determine the respective neuroprotective and neurorestorative properties of VAR in the unilateral 6-OHDA-lesioned rat and MPTP-mouse PD models, respectively. The potential beneficial effects of VAR were further examined on age-related alterations in rats, regarding antidepressant like-behavior and cognitive behavior, and hippocampal molecular signaling pathways.

2. Methods

2.1. Materials

Monoclonal antibodies against phospho-cAMP response element-binding protein (CREB) (Ser133) CREB and B cell lymphoma-2 (Bcl-2), rabbit polyclonal antibodies against phospho-(Thr202/Tyr204) and non-phospho-p44/42 extracellular signal-regulated kinases (ERK1/2), Bax, and secondary antibodies were purchased from Cell Signaling Technology Inc (Beverly, MA, USA). Monoclonal rabbit antibody against brain-derived neurotrophic factor (BDNF) was from Epitomics Inc (Burlingame, CA, USA). Polyclonal rabbit antibody against growth-associated protein (GAP)-43 was purchased from Chemicon (Tamecula, CA, USA). Mouse anti-synaptophysin monoclonal antibody, rabbit anti-tyrosine hydroxylase (TH) polyclonal antibody and goat anti-mouse IgG fluorescein isothiocyanate conjugate AP132F were purchased from Millipore (Billerica, MA, USA). Donkey anti-rabbit IgG fluorescein-conjugated antibody was from Jackson ImmunoResearch Laboratories Inc (Baltimore, MD, USA). β-actin antibody, 6-OHDA, and MPTP were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Chemical synthesis of VAR

The preparation of VAR (5-[2-(methyl-prop-2-ynyl-amino)-ethyl]-quinolin-8-ol dihydrochloride) (Ricerca Biosciences, OH, USA) is outlined in Fig. 1. The Friedel-Crafts Acylation reaction of 8-hydroxyquinoline (compound 1.1) with α-chloroacetyl chloride (compound 1.2) was carried out at room temperature (RT). Aluminum chloride ($AlCl_3$) was added to the resulting yellow suspension in small portions over 1 hour, and next heated to 100 °C for 36 hours, resulting in compound 1.3. Trifluoroacetic acid (TFA) was added to 1.3, and the reaction vessel was cooled to 0 °C. Triethylsilane (Et_3SiH) was added dropwise over 15 minutes. The reaction was gradually heated to 60 °C, resulting in compound 1.4 after 16 hours. The product was placed in a tube and suspended in acetonitrile (MeCN), followed by addition of sodium iodide (NaI) and N-propargylamine (compound 1.5). The tube was sealed under a blanket of nitrogen and heated to 100 °C for 48 hours. Hydrochloric acid (3 M in methanol) was added to the solution of VAR (compound 1.6) in dichloromethane (200 mL) and stirred at 25 °C for 1 hour. High performance liquid chromatography system and liquid chromatography-mass spectrometry analyses showed >97% purity of VAR.

2.3. Animal and treatment procedures

2.3.1. Animals

Male young C57/Bl/6J mice (2 months old) and male young (2 months old) and aged (18 months old) Sprague-Dawley rats were obtained from Harlan Laboratories, Inc, Israel. 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. For all experimental protocols, animals were weighted once a week. The average body weight was not significantly different between VAR- and vehicle-treated animals, in different experimental groups.

2.3.2. Experimental protocols and behavioral tests

For acute drug administration studies, rats were treated with VAR (7.5, 10, and 12.5 mg/kg, subcutaneous [s.c.]). Control rats were given 0.9% saline s.c. For chronic drug administration studies, mice were treated with VAR (1.1, 3.3, and 10 mg/kg, per os [p.o.], daily) for 14 days. Control mice were given deionized water p.o. The effective doses of VAR used in all experiments were chosen, based on our previous studies with the multifunctional iron-chelating compound, M30 in animal models of neurodegeneration (Gal et al., 2005; Kupershmidt et al., 2012a). One day following the last administration of the drug, the animals were sacrificed by decapitation, and striatum, hippocampus, cerebellum, liver, and small intestine have been removed rapidly and frozen in liquid nitrogen for further analyses.

6-OHDA lesion: rats were anesthetized with ketamine and/or xylazine (70/35 mg/kg, intraperitoneal injection [i.p.]) and placed in a stereotaxic frame (Kopf, CA, USA). A heating pad maintained a constant body temperature of 37 °C. The toxin 6-OHDA hydrochloride was injected into 2 locations (2 × 15 μg in 4 μL saline containing 0.1% ascorbic acid, at 1 μL/min infusion rate for 4 minutes) in the left striatum, using the following coordinates (in mm): AP, +1.2; L, ±2.5; DV,−5; and AP, +0.2, L, ±3.8, and DV, −5 with respect to the bregma. The noradrenergic neurons were protected by injecting desipramine (10 mg/kg, s.c.), 30 minutes before the 6-OHDA injection. The rats were divided into the following groups: 6-OHDA-lesioned and treated with vehicle (0.9% saline) and 6-OHDA-lesioned and treated with VAR (10 mg/kg, s.c., 3 times weekly for 3 weeks) given 1 hour before the 6-OHDA injection. The sham-operated group was subjected to all procedures,