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Effect of long-term treatment with rasagiline on cognitive deficits and related molecular cascades in aged mice

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ABSTRACT

The present study aimed to investigate the protective effects of prolonged treatment with the selective, irreversible monoamine oxidase-B inhibitor, novel anti-parkinsonian drug, rasagiline (Azilect) in aged animals. Our findings from behavioral experiments demonstrated that long-term treatment of aged mice with rasagiline (0.2 mg/kg) exerted significant beneficial effects on mood-related dysfunction and spatial learning and memory functions. At this dose of rasagiline, chronic drug administration significantly inhibited monoamine oxidase-B activity and caused an increase in striatal dopamine and serotonin levels, while decreasing their metabolism. In addition, rasagiline treatment elevated striatal mRNA expression levels of dopamine receptors D1 and D2. Furthermore, we found that rasagiline upregulated expression levels of the synaptic plasticity markers brain-derived neurotrophic factor, tyrosine kinase-B receptor, and synapsin-1, increased Bcl-2 to Bax antiapoptotic ratio and the activity of the antioxidant enzyme, catalase in brain of aged mice. The present study demonstrated that long-term treatment with rasagiline could affect behavioral deficits in aged mice and upregulate various neuroprotective parameters in the aging brain, indicating that the drug may have therapeutic potential for treatment of age-associated neurodegenerative disorders.

1. Introduction

Aging in human subjects and experimental animals is often characterized by changes in brain volume, accompanied by a decline in motor and cognitive performance (Schulze et al., 2011). In particular, the functional integrity of the hippocampus region in the brain, which plays an important role in memory formation and spatial navigation, is vulnerable to the aging process, impacting learning and memory (Driscoll and Sutherland, 2005). Brain aging is associated with a progressive imbalance between antioxidant defenses and intracellular accumulation of reactive oxygen species and free radicals (Harman, 1992; Poon et al., 2006), as well as downregulation of neurotrophic factors (Halbach, 2010), which may contribute to synaptic and cellular loss and memory deficits (Tapia-Arancibia et al., 2008).

Rasagiline (N-propargyl-1(R)-aminoindan) is an aromatic propargylamine and highly potent selective irreversible monoamine oxidase (MAO)-B inhibitor (Youdim et al., 2001), indicated for the treatment of motor symptoms in both early- and moderate-to-late stage of Parkinson's disease (PD) (Olanow et al., 2008, 2009). Several preclinical studies have demonstrated that rasagiline exerted neuroprotective effects against various neurotoxins in both cell cultures and animal PD models and in a variety of non-PD-related models (see reviews, Finberg and Gillman, 2011; Maruyama and Naoi, 2013; Naoi and Maruyama, 2010; Weinreb et al., 2010, 2011). Various molecular mechanisms appear to be associated with the neuroprotective effects of rasagiline, including upregulation of cellular antioxidant activity; induction of neurotrophic factors and neuroactive ligand receptors (Maruyama et al., 2004; Weinreb et al., 2009); prevention of the decline in mitochondrial membrane potential and nuclear translation of glyceraldehyde 3-phosphate dehydrogenase, activation of prosurvival antiapoptotic molecules (e.g., Bcl-2 and Bcl-xL), and suppression of cell death cascades initiated by proapoptotic Bcl-2 family molecules (e.g., Bax and Bad) and caspase-3 (Akao et al., 2002b; Blandini, 2005; Maruyama et al., 2001a, Weinreb et al., 2004, 2007). Moreover, it was demonstrated that rasagiline may be involved in the regulation of the molecular composition of the excitatory postsynaptic density (Gardoni et al., 2011).

Structure-activity experiments have indicated that the propargyl moiety is essential for the neuroprotective activity of rasagiline, since N-propargylamine itself was shown to promote neuronal survival via similar neuroprotective and/or neurorescue pathways

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(Bar-Am et al., 2005). Previous studies have shown that N-propargylamine induced antiapoptotic Bcl-2 family proteins reduced the expression of proapoptotic Bax and Bad and increased the availability of neurotrophic factors (Bar-Am et al., 2005; Maruyama et al., 2000b; Yogev-Falach et al., 2003). The neuroprotective activity of rasagiline was also demonstrated at concentrations below the MAO inhibition threshold (Sagi et al., 2007), as well as in cell cultures that do not contain MAO-B (Akao et al., 2002a), suggesting that the neuroprotective activity of rasagiline is not completely attributable to MAO-B inhibition and that multiple mechanisms are involved. In this regards, comparable protective effects have been also obtained with the S-enantiomer of rasagiline, which lacks MAO-B inhibitory activity (Maruyama et al., 2000a, Maruyama et al., 2001b). Additionally, the major metabolite of rasagiline, 1-(R)-aminoindan has been reported to possess antioxidant activity and neuroprotective capabilities of its own, and thus could also contribute to the overall neuroprotective activities of the parent molecule (see review, Bar-Am et al., 2010).

Previous proteomics and genomics studies in aged rats have shown that rasagiline affected various hippocampal mitochondrial genes involved in neurodegeneration, cell survival, synaptogenesis, oxidation, and metabolism (Weinreb et al., 2007). In the present study, we have further investigated the neuroprotective effects of prolonged rasagiline treatment on depressive-like behavior and spatial learning and memory impairments in normal aged mice. Additionally, the regulatory effect of rasagiline on expression of neurotrophic factors, Bcl-2 to Bax ratio and the antioxidant enzyme catalase in the hippocampus and striatum of aged mice were evaluated.

2. Materials and methods

2.1. Materials

Chemicals and reagents were of the highest analytical grade and were purchased from local commercial sources. Primers were purchased from QIAGEN Ltd (Germany). Rasagiline mesylate (N-propargyl-1R-aminoindan) was purchased from Sigma-Aldrich Co LLC (USA).

2.2. Animal treatment procedures

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. Animals were kept 3–4 per cage on a 12-hour light-dark cycle with food and water available ad libitum. The dose of rasagiline chosen on the basis of previous studies (Lamensdorf et al., 1996; Youdim et al., 2001). Aged (24-month-old) male C57Bl/6J mice were obtained from Harlan Laboratories, Inc (Israel). Rasagiline (0.2 mg/kg, per day) or vehicles (water) were orally administered daily to aged mice (7–10 mice per each experimental group) for 3 months. In parallel, young (6-month-old) mice were administered with vehicles (water, orally). In all experimental protocols, animals were weighted once a week, and no significant change in body weight was observed during treatment period. At the end of the experiment, the mice were sacrificed and brains were dissected and stored and −80 °C for further biochemical analyses.

2.3. Behavioral analyses

With the aim of assessing the effects of rasagiline on depressive-like behavioral and cognitive impairments in aged mice, behavioral studies were performed 2–3 weeks before the end of drug treatment, as followed:

2.3.1. Depressive-like behavior tests

Forced swimming test (FST) in mice, previously established as behavioral despair test (Borsini and Meli, 1988; Porsolt et al., 1977). The animals were placed individually in glass cylinders (40 cm height, 20 cm diameter) containing 20 cm depth of water at 24–26 °C. After the initial 2-minute acclimatization period, the total duration of immobility was measured for 4 minutes.

Tail suspension test (TST) in mice was performed according to the method described previously (Cryan et al., 2005). The mice were individually suspended in the hook of the TST box, 40 cm above the surface of table with an adhesive tape placed 3 quarters of the distance from the base of the tail. After 2 minutes acclimatization, immobility duration was recorded for 4 minutes.

2.3.2. Morris water maze test

Spatial learning and memory was assessed in mice, using the Morris water maze (MWM) test as previously described (Bromley-Brits et al., 2011; Morris, 1984). We used a circular tank (120 cm diameter × 50 cm height) filled to a depth of 25 cm with tepid water and a white escape platform (10 cm diameter), divided into 4 equal quadrants. The water (23 °C–26 °C) was made opaque by addition of milk. Mice were released into the water, always facing the tank wall, and given 60 seconds to find the platform. On reaching the platform, the mice were allowed to remain on it for 20 seconds. The training schedule consisted of 7 consecutive days of testing. During the 2 first days of testing, the mice were training with visible platform for three 60-second trials per day. During the 4 following days of testing, the mice were trained with hidden platform for three 60-second trials per day. Each subsequent trial was starting at a different direction for each trial. To assess memory consolidation, a probe trial is performed after the platform training trials. In this trial, the platform was removed from the tank, and mice were allowed to swim freely. For these tests, time spent in the target quadrant within 60 seconds was recorded. All trials were monitored by a video camera positioned above the pool and the behavior of each mouse is acquired by a computerized video-tracking system (Smart JUNIOR, Panlab, Spain).

2.3.3. 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. Mice were placed in the near left corner and left to explore the field freely for 5 minutes. Latency to start locomotion, line crossing, rearing, and the number of fecal pellets produced were counted (Markowska et al., 1998).

2.3.4. Object recognition test

Mice were trained and tested in the novel object recognition task, as previously described (de Lima et al., 2005, 2008). Training in the object recognition task took place in the same arena used for the open field. The object recognition test required that the mice recalled which of the 2 objects they had been previously familiarized with. Twenty-four hours after arena exploration, training was conducted by placing individual mouse into the field, in which 2 identical objects (objects A1 and A2) were positioned in 2 adjacent corners. Mice were left to explore the objects until they had accumulated 30 seconds of total object exploration time or for a maximum of 20 minutes. In a short-term memory test, given 1.5 hours after training, the mouse explored the open field for 5 minutes in the presence of 1 familiar and 1 novel object. In a long-term memory test, given 24 hours after training, the same mouse