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Research Paper

Treatment with the MAO-A inhibitor clorgyline elevates monoamine neurotransmitter levels and improves affective phenotypes in a mouse model of Huntington disease

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ABSTRACT

Abnormal monoamine oxidase A and B (MAO-A/B) activity and an imbalance in monoamine neurotransmitters have been suggested to underlie the pathobiology of depression, a major psychiatric symptom observed in patients with neurodegenerative diseases, such as Huntington disease (HD). Increased MAO-A/B activity has been observed in brain tissue from patients with HD and in human and rodent HD neural cells. Using the YAC128 mouse model of HD, we studied the effect of an irreversible MAO-A inhibitor, clorgyline, on the levels of select monoamine neurotransmitters associated with affective function. We observed a decrease in striatal levels of the MAO-A/B substrates, dopamine and norepinephrine, in YAC128 HD mice compared with wild-type mice, which was accompanied by increased anxiety- and depressive-like behaviour at five months of age. Treatment for 26 days with clorgyline restored dopamine, serotonin, and norepinephrine neurotransmitter levels in the striatum and reduced anxiety- and depressive-like behaviour in YAC128 HD mice. This study supports a potential therapeutic use for MAO-A inhibitors in the treatment of depression and anxiety in patients with HD.

1. Introduction

Monoamine oxidases (MAO) play an important role in brain function via the metabolic regulation of monoamine neurotransmitters, such as dopamine (DA), norepinephrine (NE), and serotonin (5-HT). Alterations in MAO activity, which are associated with changes in monoamine neurotransmitter levels as well as with the production of toxic reactive oxygen species, have been implicated in the pathobiology of neuropsychiatric and neurodegenerative disorders (Duncan et al., 2012). Interestingly, psychiatric manifestations are a common feature of many neurodegenerative disorders including Parkinson's (PD) and Huntington disease (HD). Indeed, depression is the most prevalent symptom in PD and HD, occurring in approximately 40–60% of patients (Paulsen et al., 2005; Youdim and Bakhle, 2006).

Two isoforms of MAO exist, MAO-A and MAO-B, which share approximately 70% amino acid identity. Both proteins are expressed in most mammalian tissues, associated with the outer membrane of the mitochondria (Finberg, 2014; Youdim et al., 2006); however, the ratio of MAO-A and MAO-B isoforms and the levels of MAO activity vary between regions of the human brain (Youdim et al., 2006). Enzymatically, MAO-A and MAO-B differ in their substrate selectivity. NE and 5-HT are specific substrates for MAO-A, whereas phenylethylamine (PEA) and benzylamine are only degraded by MAO-B. DA is a common substrate for both isozymes (Duncan et al., 2012).

Abnormal MAO-A and MAO-B activity are therefore involved in distinct clinical presentations (Duncan et al., 2012). Dysregulation in MAO-A activity has been implicated in a variety of neuropsychiatric disorders including depression, anxiety, autism, and attention deficit hyperactivity disorder, whereas MAO-B activity, which has been described to increase with ageing, is associated with neurodegenerative disorders, such as PD (Duncan et al., 2012). Moreover, alterations in both MAO-A and B activity have been observed in brain regions that undergo neurodegeneration in HD patients, such as the basal ganglia and the pons (Richards et al., 2011).

Monoamine oxidase inhibitors have long been used for treatment of psychiatric disorders and, more recently, have shown therapeutic benefits in the treatment of neurodegenerative disorders (Youdim and Bakhle, 2006). MAO inhibitors can be classified as reversible or

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irreversible inhibitors of MAO-A, MAO-B, or both (Finberg, 2014; Youdim et al., 2006). Inhibition of MAO-A results in antidepressant and anxiolytic effects (Cryan and Holmes, 2005; Finberg, 2014), whereas selective MAO-B inhibitors are useful in movement disorders such as PD (Finberg, 2014; Youdim and Bakhle, 2006). Although MAO inhibitors have yet to be tested in the treatment of HD, the presence of psychiatric manifestations and increased MAO-A and MAO-B activity in HD patients suggest that MAO inhibitors may be of therapeutic benefit.

Recently, we showed that inhibition of excessive MAO activity in mouse and human HD neural cells using clorgyline, an irreversible MAO-A inhibitor, reduces oxidative stress and improves cellular viability (Ooi et al., 2015). These observations prompted us to evaluate the effects of clorgyline further in the YAC128 (FVB/N) mouse model of HD. The YAC128 HD mice express a full-length human mutant *HTT* transgene and exhibit neuropathological and behavioural phenotypes that mimic symptoms of patients with HD, including affective phenotypes such depressive- and anxiety-like behaviour (Pouladi et al., 2013; Slow et al., 2003). After establishing the appropriate dose of clorgyline in wild-type (WT) FVB/N mice, we sought to determine the effect of MAO-A inhibition on monoamine neurotransmitter levels and affective phenotypes in YAC128 HD mice.

2. Material and methods

2.1. Animals

Three-month-old male FVB/N mice, purchased from InVivos (Singapore), used for the initial clorgyline dosing study, and four-month-old YAC128 HD and littermate wild-type mice were group-housed on a reverse 12-h light/dark cycle. The mice had ad libitum access to water and food throughout the study. Clorgyline hydrochloride (Sigma) was diluted in phosphate buffered saline (PBS) and was delivered by intraperitoneal injection (i.p.) once per day. The diluted drug solution was free of precipitates. All animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the Biological Resource Centre, ASTAR.

2.2. Drug treatment

For the clorgyline dosing study, 3 month old WT mice were divided into four groups of 10 mice each. Three groups received clorgyline at a dose of 0.5, 1.5, or 3 mg/kg and the fourth group received an equivalent volume of PBS. For the YAC128 treatment study (Fig. 1A), three independent cohorts of 4 month old mice were used, giving a total of 17–22 mice per treatment/group. For each cohort, mice were divided into three groups. One group of YAC128 HD mice received clorgyline at a dose of 1.5 mg/kg and two groups, one of WT mice and another one of YAC128 HD mice, received an equivalent volume of PBS (Fig. 1B). Treatments were administered daily before noon at a volume of 10 mL/kg i.p. for 21 days and were continued throughout the behavioural testing phase, which commenced on day 22. During the behavioural testing phase, mice were treated in the afternoon following completion of the corresponding behavioural test. Mice were sacrificed once the behavioural testing was finished at day 26 or 28 (Fig. 1).

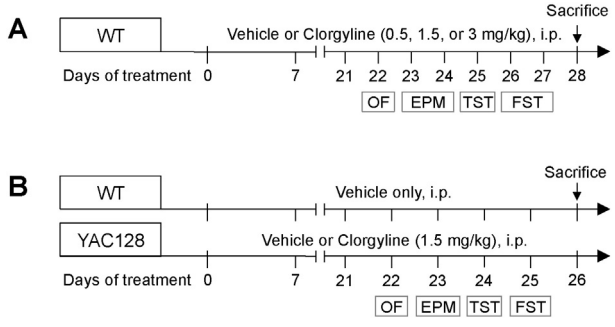

Fig. 1. Schematic representation of the experimental design for the clorgyline dosing study in WT mice and efficacy study in YAC128 HD mice. (A) WT mice were injected intraperitoneally (i.p.) with vehicle (PBS) or clorgyline (0.5, 1.5, or 3 mg/kg) daily for 28 days. (B) WT mice were injected i.p. with vehicle (PBS) only, whereas YAC128 HD mice were injected with either vehicle (PBS) or clorgyline (1.5 mg/kg), daily for 26 days. For both (A) and (B), behavioural tests commenced on day 22 and were carried out in this order: open field (OF), elevated plus maze (EPM), tail suspension test (TST), and the forced swim test (FST). Mice were sacrificed on day 26 or 28 and brain tissue was harvested for biochemical assessments.

2.3. Determination of MAO-A activity in brain tissue

The MAO-Glo Assay System (Promega) was used to measure MAO-A activity. Brain tissue was homogenized in lysis buffer (50 mM TRIS pH 8.0, 150 mM NaCl, 1% Igepal, 1× Roche complete proteinase inhibitor, 0.005 mM ZVAD, and 1 mM phenylmethulsulfonyl fluoride in Milli-Q water). Protein lysates were diluted to 1 mg/mL using lysis buffer. Diluted lysate (25 μL) was incubated with an equal volume of MAO substrate solution (1:25 dilution of provided MAO substrate) for 2 h at room temperature. Luciferin detection reagent (50 μL) was added and luminescence was measured using FLUOstar Omega plate reader (BMG Labtech). Except for the lysis buffer, all solutions and reagents were provided by the MAO-Glo Assay System (Promega).

2.4. Determination of dopamine, norepinephrine, serotonin, and DOPAC levels in brain tissues

Following completion of behavioural testing, mice were sacrificed by carbon dioxide asphyxiation followed by rapid removal of the brains. Brains were micro-dissected on ice and immediately snap-frozen in liquid nitrogen. DA, NE, and 5-HT were determined by Brains On-line (Groningen, Netherlands) using established methods. Briefly, for the preparation of LC–MS samples, 6 mL of 0.5 M perchloric acid was added to each mg of striatal tissue and the samples were homogenized by sonication. The homogenates were centrifuged and the supernatants were stored as brain extracts at −80 °C until analysis. For analysis, an aliquot of internal standard solution was mixed with a diluted aliquot of each brain extract sample. The mixture was centrifuged and the supernatant was transferred to a vial suitable for use in the autosampler. Concentrations of 5-HT, DA, NE, and DOPAC were determined by HPLC with tandem mass spectrometry (MS/MS) detection, using deuterated internal standards of the analytes. For each LC–MS sample, an aliquot was injected onto the HPLC column by an automated sample injector (SIL10-20 AC-HT, Shimadzu, Japan). Chromatographic separation was performed on a SynergiMax column (100 × 3.0 mm, particle size 2.5 μm) held at a temperature of 35 °C. The mobile phases consisted of A: ultra-purified H_2O + 0.1% formic, and B: acetonitrile: ultra-purified H_2O (70:30) + 0.1% formic acid. Elution of the compounds proceeded using a suitable linear gradient at a flow rate of 0.3 mL/min. The MS analyses were performed using an API 4000 MS/MS system consisting of an API 4000 MS/MS detector and a Turbo Ion Spray interface (Applied Biosystems, the Netherlands). The acquisitions on API 4000 were performed in positive ionisation mode, with optimised settings for the analytes. The instrument was operated in multiple-reaction-monitoring (MRM) mode. Data were calibrated and quantified using the Analyst data system (Applied Biosystems, version 1.6.2, Netherlands). Concentrations in experimental samples were calculated based on the calibration curve in the corresponding matrix.

2.5. Behavioural tests of affective function

All behavioural tests were performed in the morning during the dark phase of the reverse light/dark-cycle.