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Research Report

The dopaminergic stabilizer, (–)-OSU6162, rescues striatal neurons with normal and expanded polyglutamine chains in huntingtin protein from exposure to free radicals and mitochondrial toxins

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disease characterized by progressive motor, cognitive and psychiatric deficits, associated with predominant loss of striatal neurons and caused by a polyglutamine expansion in the huntingtin protein. There is so far neither cure nor approved disease-slowing therapy for HD, though recent clinical studies have shown a beneficial long-term effect of pridopidine in patients with HD. The nature of this effect, purely symptomatic or, in addition, neuroprotective, is difficult to elucidate in clinical trials. Pridopidine and (–)-OSU6162 are members of a new family of compounds referred to as dopaminergic stabilizers, which normalize abnormal dopamine neurotransmission.

We investigated the effects of (–)-OSU6162 on huntingtin knocked-in striatal neurons in culture. Control neurons had normal full-length huntingtin with 7 glutamines while "mutant" neurons had large expansions (Q=111). We studied the dose–effect curves of (–)-OSU6162 on mitochondrial activity, LDH levels, necrosis and apoptosis in untreated Q7 and Q111 cells. In addition, we investigated the effects of (–)-OSU6162 on Q7 and Q111 neurons challenged with different neurotoxins such as sodium glutamate, H_2O_2, rotenone and 3-nitropropionic acid (3NP). As we found prevention of toxicity of some of these neurotoxins, we investigated the putative neuroprotective mechanisms of action of (–)-OSU6162 measuring the effects of this dopaminergic stabilizer on expression and release of BDNF, the ratios of Bcl2/Bax proteins and of p-ERK/ERK, the levels of chaperones and GSH, and the effects of (–)-OSU6162 on dopamine uptake and release.

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Abbreviations: BDNF, brain derived neurotrophic factor; CHIP, carboxy terminus of Hsp70-binding protein; DIV, days in vitro; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; GSH, glutathione; HD, Huntington's disease; HSP70, heat-shock protein 70 kDa; H_2O_2, hydrogen peroxide; LDH, lactate dehydrogenase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; 3NP, 3-nitropropionic acid; p-ERK, phospho-extracellular signal-regulated kinase; Q7, $STHdH^{Q7/Q7}$ cells; Q111, $STHdH^{Q111/Q111}$ mutant cells

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We found that (–)-OSU6162, 3–150 μM, produces a dose dependent increase of mitochondrial activity and a reduction of cell death. (–)-OSU6162 does not change glutamate toxicity, but it partially prevents that of H_2O_2, rotenone and 3-nitropropionic acid. (–)-OSU6162 increases the intracellular levels of BDNF and Bcl2/Bax and decreases those of p-ERK/ERK and CHIP in Q111 cells. (–)-OSU6162 increased ^{3}H-dopamine uptake and amphetamine-induced ^{3}H-dopamine release in E13 mouse mid brain neurons.
Our studies demonstrate that (–)-OSU6162 improves survival and mitochondrial function in striatal Q111 neurons and the resistance of these cells to several striatal neurotoxins, suggesting that (–)-OSU6162 and related compounds should be tested for neuroprotection in animal models and, eventually, in patients with HD.

1. Introduction

Huntington's disease (HD) is an autosomal dominant, neurodegenerative disease caused by an expanded CAG repeat chain in the huntingtin gene (Walker, 2007). Its clinical features include a range of motor, cognitive and behavioral deficits and its neuropathological findings are characterized by predominant cell loss in the caudate and putamen, particularly medium spiny striatal neurones (Walker, 2007).

With the exception of the effectiveness of tetrabenazine on chorea, there is no treatment with global symptomatic or disease-modifying beneficial effect in HD (Mestre et al., 2009a, 2009b). A recent phase III, multinational, 6-month, randomized, double-blind, placebo-controlled trial designed to assess the symptomatic effects of pridopidine suggested to us that the beneficial effects of pridopidine on motor deficits improve over time (de Yebenes et al., 2011; Lundin et al., 2010). Also, during the period of 26 weeks of observation, there was no deterioration of the motor scores in the pridopidine (45 mg bid)-treated patients, while there was a worsening of the motor scores in the placebo-treated group, raising the question of a putative disease-modifying effect of pridopidine, beyond the symptomatic effect on motor symptoms in HD (de Yebenes et al., 2011; Lundin et al., 2010).

(–)-OSU6162 and pridopidine are members of a novel class of compounds called dopaminergic stabilizers (Natesan et al., 2006; Ponten et al., 2010; Rung et al., 2008; Sonesson et al., 1994), which act primarily on dopamine (DA) type 2 (D_2) receptors, enhancing or reducing dopamine-dependent effects according to the initial level of activity. (–)-OSU6162 is a partial agonist at both dopamine D2 receptors and 5-HT2A receptors and has a higher binding affinity to D2 but is a weaker partial agonist at 5-HT2A receptor (Burstein et al., 2011; Carlsson et al., 2011; Dyhring et al., 2010; Kara et al., 2010; Natesan et al., 2006). In vivo, these compounds stabilize dysregulated psychomotor functions while having only subtle effects on normal psychomotor activity (Dyhring et al., 2010; Natesan et al., 2006; Pettersson et al., 2010; Ponten et al., 2010; Seeman et al., 2009; Sonesson et al., 1994).

There are several putative mechanisms through which dopamine stabilizers could have neuroprotective effects in models of HD and in patients with this disease. The preclinical in vivo pharmacological action of dopaminergic stabilizers, have shown that these compounds have a direct effect on dopamine neurotransmission and dopamine receptors, and an indirect effect on glutamate neurotransmission mostly consistent with the regulation of glutamate release (Carlsson et al., 2004) without excluding downstream effects on NMDA receptor-mediated effects (Waters et al., 2009). Some of the pharmacological effects of dopamine stabilizers on dopamine neurotransmission could be neurotrophic or neuroprotective for the striatal neurons since they interact with mechanisms involved in cell survival (Hardingham and Bading, 2010). Recent studies have shown that both the dopamine metabolite di-hydroxy-phenyl-acetic acid (DOPAC), alone or coupled with nitric oxide (NO), reduces levels of GSH and the activity of mitochondrial complex I (Nunes et al., 2011). In addition, other dopamine derivatives, such as quinone oxidation products of dopamine, interfere with mitochondrial function (Jana et al., 2011), an abnormality that is observed in patients with HD and in models of this disease.

These data may suggest that dopaminergic stabilizers could have beneficial effects on disease mechanisms in neurodegenerative states and in diseases such as HD. It is, however, very difficult to differentiate true neuroprotection from long-term symptomatic effects in clinical trials. We, therefore, investigated the putative neuroprotective properties of (–)-OSU6162 in huntingtin mutant knocked-in striatal cells in vitro.

2. Results

2.1. *Dose–response curve of the effects of (–)-OSU6162 on STHdhQ7 and Q111 cells*

The dose–response curve with (–)-OSU6162 at concentrations from 3 to 150 μM in Q7 and Q111 cells revealed that there is a dose-dependent increase of the mitochondrial activity in both Q7 and Q111 cells, in the range of concentrations of (–)-OSU6162 tested in these experiments (Fig. 1A). Untreated Q111 cells had higher levels of LDH than Q7 cells, suggesting a greater rate of necrosis in cells with large polyglutamine expansions in huntingtin protein. (–)-OSU6162 also produced a dose-related reduction of LDH levels in Q111 cells in the range of doses investigated (Fig. 1B) as well as a reduction in the number of trypan blue (Figs. 1C and D) and in the number of propidium iodide positive cells (Figs. 1E and F). Q111 cells were more sensitive to serum deprivation than Q7 cells and (–)-OSU6162 protected from the cell death induced by serum deprivation.