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Protective effects of isolecanoric acid on neurodegenerative in vitro models

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ABSTRACT

Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS), are neurodegenerative disorders characterized by loss of dopaminergic or motor neurons, respectively. Although understanding of the PD and ALS pathogenesis remains incomplete, increasing evidence from human and animal studies has suggested that aberrant GSK3β, oxidative stress and mitochondrial damage are involved in their pathogenesis. Using two different molecular models, treatment with L-BMAA for ALS and rotenone for PD the effect of isolecanoric acid, a natural product isolated from a fungal culture, was evaluated. Pre-treatment with this molecule caused inhibition of GSK3β and CK1, and a decrease in oxidative stress, mitochondrial damage, apoptosis and cell death. Taken together, these results indicated that isolecanoric acid might have a protective effect against the development of these neurodegenerative disorders.

1. Introduction

Glycogen synthase kinase-3 (GSK3) is a ubiquitously expressed protein kinase that exists in two isoforms, α and β. The β isoform has an established role in cell survival and viability. GSK3β is involved in the regulation of a wide range of cellular functions including differentiation, growth, proliferation, motility, cell cycle progression, embryonic development, apoptosis and insulin response.

Aberrant GSK3β activity has been linked with several human diseases including neurodegenerative and psychiatric disorders (Eldar-Finkelman, 2002; Doble and Woodgett, 2003; Hooper, et al., 2008; Hur, E.M. and Zhou, F.Q., 2010) GSK-3β activity inhibition is therefore proposed to have a therapeutic benefit in the treatment of these diseases, and intensive efforts have been made in this area, focused on searching new GSK3B inhibitors or design better ones.

Unlike other protein kinases, GSK3β is constitutively active in resting conditions and is inhibited in response to upstream signals. It can be inhibited or over-activated by diverse post-transductional modifications such as phosphorylation in response to upstream signals (Eldar-Finkelman, 2002) Phosphorylation of GSK3β downstream targets typically results in attenuation of the signaling pathway and/or inhibition of the substrate's activity. In neuron, GSK3β is intimately involved with the control of apoptosis, synaptic plasticity, axon formation and neurogenesis (Jiang et al. 2005; Kim et al., 2006)).

Some in vivo studies indicate that over-activity of GSK3β results in adverse effects. This over-activity should be produced by an increase in GSK3β expression or by an imbalance of its phophorylation state leading to a super-active enzymatic state (Eldar-Finkelman, 2002).

In this paper we have focused on the involvement of GSK3β in two neurodegenerative diseases, ALS and Pakinson's disease (PD)

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and the effect of a newly encountered GSK3β inhibitor (isolecanoric acid) on these diseases.

Parkinson's disease is characterized by dopaminergic neuron degeneration in the substantia nigra pars compacta (SNpc) with Lewy body (LB) pathology, accompanied by clinically defined Parkinsonism (Cookson, M.R., 2005). Because of the potential role of tau in PD (Lei et al., 2010; Simon-Sanchez et al., 2009; Edwards et al., 2010), the function of GSK3β in PD has also been investigated. The examination of postmortem tissue from PD patients has revealed that GSK3β, phosphorylated at Ser9, is specifically localized within the halo of LBs (Nagao and Hayashi, 2009) and that GSK3β activity is also elevated in the striatum (Wills et al., 2010). This finding has been observed in mouse models of PD (Duka, et al., 2009). Increased GSK3β levels have also been reported in peripheral blood lymphocytes in PD patients (Armentero, et al., 2011), and polymorphisms in GSK3β, which affect its transcription and splicing, are also associated with disease risk in PD when stratifying by tau haplotype (Kwok, et al., 2005; Garcia-Gorostiaga, et al., 2009).

Mechanistically there is evidence to support an interaction between α-synuclein, a 16 kDa natively unstructured protein that is fundamentally involved in the pathogenesis of PD, and GSK3β. Aggregated α-synuclein species are the main components of LBs, and single nucleotide polymorphisms and duplication or triplication of the α-synuclein gene cause familial Parkinsonian degeneration (Cookson, M.R., 2005; Wills, et al., 2010). α-synuclein, which is a substrate for GSK3β phosphorylation, may also modulate the activation of GSK-3β (Khandelwal, et al., 2010). The potential role of GSK3β in PD has been elucidated in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD, where the inhibition of GSK3β protects against MPTP toxicity in vitro and in vivo (Kozikowski, et al., 2006; Duka, et al., 2009; Wang, et al., 2007) and decreases α-synuclein protein expression (Kozikowski, et al., 2006). These data strongly implicate GSK3β in the pathogenesis of PD.

Previous studies have reported that GSK3β increases abnormally in vitro and in vivo models of ALS, in the thoracic spinal cord tissue of patients with sporadic ALS (Hu, et al., 2003), and in the frontal and temporal cortices of ALS patients. Moreover, GSK3β suppression attenuates disease progression in an ALS mouse model (Yang, et al., 2008; Sugai, et al., 2004; Feng, et al., 2008). Many studies have shown that GSK3β inhibition can suppress disease progression of ALS in both in vitro and in vivo models. Caldero et al. (2010) reported that lithium, a GSK3β inhibitor, prevents the excitotoxic cell death of motor neurons. Feng et al. (2008) also reported that disease onset, disease progression, and survival in ALS mouse models were prolonged by the treatments of specific inhibitors of GSK3β or materials with GSK3β inhibitor effects.

GSK3β participates in different processes by regulating the mitochondria and inducingapoptosis which impacts regulating different proteins implicated in the process. Italso participates in the ER stress process, and GSK3β inhibition leads to the activation of different antioxidative pathways via Nrtf2 gene transcription. At the same time, oxidative stress is a common process that directly or indirectly kills the motoneurons. So, the interaction with this pathway, either by indirectly stimulating GSK3β and the Nrft-2 antioxidant gene transcription, or by actuating directly as a scaffold for the free radicals generated, was reduced the oxidative stress and the death of motoneurons.

In this paper, the effect of a GSK3β inhibitor isolated from fungus *Glarea lozoyensis*, isolecanoric acid, has been studied in these two neurodegenerative diseases. The β-N-methylamino-L-alanine (L-BMAA) toxicity method to mimic the territorial ALS and damage with rotenone were used as a model of PD. Both methods have been employed to emulate these diseases in animal models.

2. Materials and methods

2.1. Cell culture

SH-SY5Y cells, a human dopaminergic neuroblastoma cell line, were obtained from an American Type Culture Collection (Rockville,MD, USA) and grown in DMEM + F12 supplemented with 10% FBS, 2 mM L-glutamine, penicillin/streptomycin (100 U/mL), 1 mM sodium pyruvate and 0.1 mM non-essential amino acids (Invitrogen) at 37 °C under a humidified atmosphere of 95% air and 5% CO_2. In all assays, the test compounds were dissolved in dimethyl sulfoxide (DMSO) and added to the cells at a final concentration of 0.5% (v/v) DMSO. Previous DMSO curves indicated that no significant cytotoxicity was observed at this concentration (data not shown).

2.2. Protein determination

Total protein concentrations in the SH-SY5Y cells were determined using Pierce™ BCA Protein Assay Kit (Thermo Scientific) according to the protocol of the manufacturer with albumin as the standard.

2.3. Strain and fermentation

The fungus *Glarea lozoyensis* CF-160870, ATCC-20868 (Ascomycota, Leotiomycetes) (Bills, et al. 1999 is used to produce pneumocandin B_0, the starting molecule for the synthesis of the antifungal drug caspofungin (CANCIDAS™) (Peláez et al. 2011).

To scale up the fermentation to 1 L, 10 mycelial discs were used to inoculate 50 mL of SMYA (Difco neopeptone 10 g, maltose 40 g, Difco yeast extract 10 g, agar 4 g, distilled H_2O 1 L). After 7 days incubation at 22 °C and 220 rpm, 1.5 mL aliquots of this culture were used to inoculate MV8 medium (maltose 75 g, V8 juice 200 ml, Soybean I (Sigma–Aldrich) 1 g, L-proline 3 g, MES 16.2 g; 1 L distilled H_2O) distributed among 20 × 50 mL in 250 mL Erlenmeyer flasks. The flasks were incubated at 22 °C, 220 rpm and 70% relative humidity for 14 days.

2.4. Isolation and structural elucidation of isolecanoric acid

A 1 L culture of *Glarea lozoyensis* in MV8 medium was extracted by the addition of acetone (1 L), shaking for 1 h, centrifugation at 8500 rpm for 5 min and filtration under vacuum. The remaining liquid was evaporated under a nitrogen stream to a final volume of 1 L and filtered through Celite. The filtered solution was loaded onto a SP207ss column (65 g) with the continuous addition of water and the column was eluted with a water: acetone gradient (8 mL/min; 10–100 acetone in 12.5 min; 100% acetone for 15 min).

Fractions were dried and analyzed by HPLC-MS and tested for GSK-3β inhibitory activity. Bioactive fractions were subjected to a second chromatography on Sephadex LH-20 (22 g; 100% methanol,

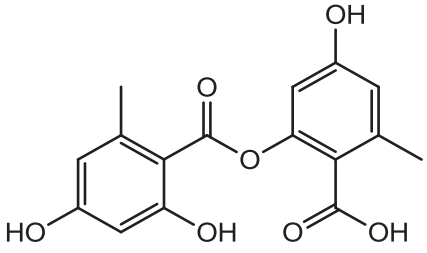

Fig. 1. Structure of isolecanoric acid.