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Research Report

Early use of oleanolic acid provides protection against 6-hydroxydopamine induced dopamine neurodegeneration

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ABSTRACT

Oleanolic acid is a triterpenoid that has been shown to possess antioxidant properties. In this study we investigated the effects of oleanolic acid in a parkinsonian rat model. Unilateral 6-hydroxydopamine (6-OHDA) lesions were carried out on postnatal day (PND) 60 in 4 groups viz. (1) Rats that started oleanolic acid treatment 7 days prior to lesion. (2) Rats not treated with oleanolic acid. (3) Rats that started oleanolic acid treatment 1 day post-lesion. (4) Rats treated with oleanolic acid 7 days post-lesion. The degree of forelimb impairment was assessed using limb use asymmetry and forelimb akinesia tests. Neurochemical changes were assessed using a Dopamine ELISA kit and mitochondrial apoptosis was measured using a mitochondrial apoptosis detection kit. In this study, animals injected with 6-OHDA displayed forelimb use asymmetry that was ameliorated by treatment with oleanolic acid 7 days pre- and 1 day post-lesion. In the cylinder test, rats injected with 6-OHDA favored using the forelimb ipsilateral (unimpaired) to the lesioned hemisphere while rats treated with oleanolic acid used the forelimb contralateral (impaired) to the lesioned hemisphere significantly more. Rats treated with oleanolic acid 7 days pre- and 1 day post-lesion had more dopamine in the striatum than the non-treated or the 7 days after lesion rats. Similarly, 6-OHDA-induced membrane depolarization was decreased in rats that received oleanolic acid treatment pre- or immediately post-lesion. This suggests that early treatment with oleanolic acid protects dopamine neurons from the toxic effects of 6-OHDA.

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1. Introduction

The neurotoxicity of 6-hydroxydopamine (6-OHDA) has been shown to be initiated by extracellular auto-oxidation which leads to the generation of oxidative products which result in the induction of oxidative stress (Ferro et al., 2005; Yuan et al., 2005). 6-OHDA can also induce neuronal cell death by blocking complex I in the mitochondria, thereby uncoupling the mitochondrial electron transport chain and reducing the electrochemical gradient (Rodriguez-Pallares et al., 2009). This leads to a decrease in the production of ATP and an increase in the levels of free radicals (Blum et al., 2001). Increasing levels of these free radicals alter homeostatic balances between reactive oxygen species and antioxidants leading to oxidative stress (Hara et al., 2003; Goes et al., 2014).

The integrity of dopamine neurons in the basal ganglia is imperative for normal functioning of the motor as well as the non-motor systems (reward and motivation) (Hemmerle et al., 2012). Parkinson's disease which affects approximately 1% of people over the age of 65 years is progressive in nature (Redgrave and Vautrelle, 2011). Degeneration of the majority of dopamine neurons in the substantia nigra pars compacta of the basal ganglia results in the subsequent impairment of motor functions producing symptoms such as bradykinesia, akinesia, postural instability, muscular rigidity and resting tremor (Chaturvedi and Beal, 2008). A hemiparkinsonian rat model can be created by injecting the neurotoxin 6-OHDA into the medial forebrain bundle (Debeir et al., 2005; Ferro et al., 2005). This neurotoxin creates a progressive animal model which mimics the progressive nature of the disease in humans (Yuan et al., 2005).

Invasive and non-invasive forms of therapeutic treatments such as deep brain stimulation, glial cell-line derived neurotrophic factor (GDNF) releasing cell transplant, L-Dopa and exercise are used to alleviate the symptoms of Parkinson's disease (Lloyd et al., 1975; Alexi et al., 2000; Frank et al., 2007; Kern and Kumar, 2007). However, their effects are short-lived and possess side effects (Howells et al., 2005; Frank et al., 2007). These treatments tend to exert their effects on the symptoms rather than providing neuroprotection (Maranis et al., 2011). As the generation of reactive oxygen species has been shown to play a major role in the degeneration of dopamine neurons in animal models for Parkinson's disease, it is imperative that new treatment modalities focusing on treatments that possess anti-oxidant properties should be considered.

Oleanolic acid is a pentacyclic triterpenoid isolated from more than 1620 plant species including Syzygium Aromaticum (Cloves) (Liu, 1995; Pollier and Goossens, 2012). This triterpenoid has been widely utilized in most parts of Asia for treating various ailments (Wang et al., 2010). Oleanolic acid has been shown to possess anti-inflammatory, anti-tumour, hepatoprotective, hypoglycemic, anti-hyperlipidemic and anti-oxidant properties (Liu, 1995; Hsu et al., 2006; Tong et al., 2011). Inhibition of reactive oxygen species formation protects against cell death (Wang et al., 2010). It has also been shown that oleanolic acid inhibits the formation of reactive oxygen species in the brain of a rat model of Alzheimer's disease (Cho et al., 2009), therefore making oleanolic acid a candidate for treating diseases resulting from an increase in reactive oxygen species such as Parkinson's disease.

In this study we looked at whether oleanolic acid use can protect against the neurotoxic effects of 6-OHDA in a parkinsonian rat model and whether this protection involved attenuating 6-OHDA-induced mitochondrial membrane depolarization.

2. Results

2.1. Step test

There was an oleanolic acid (OA) effect as the average step length of the forelimb contralateral to the lesioned hemisphere of rats treated with oleanolic acid 7 days prior and 1 day post 6-OHDA lesion was significantly shorter than that of the 6-OHDA only group ⁎ (6-OHDA vs. OA 7 days prior, OA 1 day after, $p<0.05$, Fig. 1).

2.2. Cylinder test

Exposure of rats to oleanolic acid 7 days prior, 1 day post and 7 days post-lesion attenuated the limb use asymmetry effect of 6-OHDA * (6-OHDA only vs. OA 7 days prior, $p<0.05$; 6-OHDA only vs. OA 1 day after, $p<0.05$; 6-OHDA only vs. OA 7 days after, $p<0.05$, Fig. 2).

2.3. Dopamine concentration

Striatal dopamine concentration quantification showed a 6-OHDA effect as there was a decrease in the concentration of dopamine in the striatum of the lesioned (left) hemisphere when compared to the non-lesioned (right) hemisphere * (lesioned vs. non-lesioned, $p<0.05$, Fig. 3a).

Treatment with oleanolic acid 7 days prior and 1 day post-lesion attenuated 6-OHDA-induced dopamine decrease on the lesioned (left) hemisphere * (6-OHDA only vs. OA 7 days pre- and OA 1 day after, $p<0.05$, Fig. 3b).

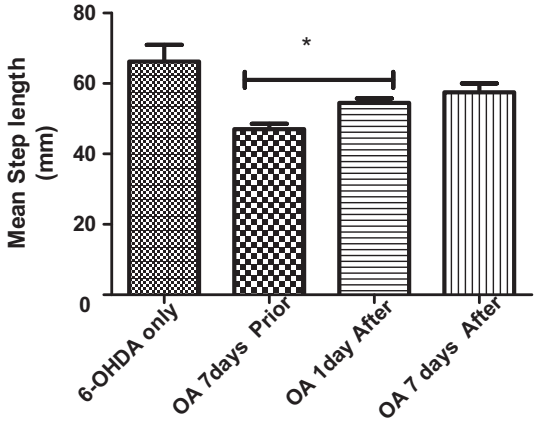

Fig. 1 – Average step length of the forelimb contralateral to the lesioned hemisphere of rats injected with 6-OHDA (6-OHDA Only, $n=10$). Step length was also measured in rats treated with oleanolic acid 7 days pre-lesion (OA 7 days prior, $n=10$), 1 day post-lesion (1 day after, $n=10$) and 7 days post-lesion (OA 7 days after, $n=10$). * (6-OHDA Only vs. OA 7 days prior, OA 1 day after, $p<0.05$).