

# Neuroprotective effects of oxyresveratrol on 6-hydroxydopamine on medial forebrain bundles in a rat model of Parkinson disease: abridged secondary publication

A Shah, YS Ho, KM Ng, M Wang, C Legido-Quigley, RCC Chang \*

## KEY MESSAGES

1. Oxyresveratrol significantly minimises motor deficits examined by apomorphine-induced rotation test, cylinder asymmetric test, and rotarod test.
2. However, oxyresveratrol cannot significantly reduce the loss of dopaminergic neurons. This suggests that oxyresveratrol per se may not be sufficient to elicit full protection.

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<sup>1,2</sup> A Shah, <sup>3</sup> YS Ho, <sup>4</sup> KM Ng, <sup>5</sup> M Wang, <sup>2,6</sup> C Legido-Quigley, <sup>1,7</sup> RCC Chang

<sup>1</sup> Laboratory of Neurodegenerative Diseases, School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong

<sup>2</sup> Institute of Pharmaceutical Science, Faculty of Life Sciences and Medicine, King's College London, UK

<sup>3</sup> School of Nursing, Faculty of Health and Social Sciences, Hong Kong Polytechnic University

<sup>4</sup> Department of Chemistry, Faculty of Sciences, The University of Hong Kong

<sup>5</sup> School of Biological Sciences, Faculty of Sciences, The University of Hong Kong

<sup>6</sup> Steno Diabetes Center Copenhagen, Gentofte, Denmark

<sup>7</sup> State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong

\* Principal applicant and corresponding author: rccchang@hku.hk

## Introduction

Nutraceuticals or natural products play protective roles in different neurodegenerative diseases, partly because of their antioxidant properties. These products exhibit prophylactic properties to prevent and delay disease progression. Resveratrol, curcumin, coenzyme Q10, vitamin E, and alpha-lipoate have protective effects in Parkinson disease (PD), but there are no detailed studies about their permeability of the blood-brain barrier (BBB). Therapies for neurodegenerative disease should ideally have high BBB permeability for optimal effects. Oxyresveratrol (OXY) has an enhanced ability to cross the BBB in an in vivo model.<sup>1</sup> OXY is a hydroxyl derivative of resveratrol (RES). It is a stilbene isolated by the hydrolytic activation of Mulberroside A, a compound found primarily in the root of *Morus Alba* (white mulberry). OXY can provide neuroprotective effects against  $\beta$ -amyloid peptide in cultured cortical neurons and hippocampal neurons after cerebral ischaemia by middle cerebral occlusion. OXY can protect neurons from an in vitro traumatic injury model and H<sub>2</sub>O<sub>2</sub>-induced PC12 cell death. There are enhanced antioxidant effects of OXY over RES. We have shown the neuroprotective effects of OXY on reducing apoptosis in the SH-SY5Y cell line in a PD model.

PD is characterised as the progressive degeneration of a selective group of dopaminergic

(DA) neurons in the substantia nigra pars compacta. Its incidence rate is markedly increased with age, with symptoms including tremor, rigidity, and problems with voluntary movement. Parkinsonian mimetics such as 1-methyl-4-phenylpyridinium and 6-hydroxydopamine (6-OHDA) provide valuable experimental models and preclinical platforms to investigate neuroprotective drugs from nutraceuticals and natural products. We aimed to use a 6-OHDA animal model to investigate the effects of OXY on motor deficits and the neuronal loss of dopaminergic neurons.

Common sites for intracerebral injection of 6-OHDA are the striatum, substantia nigra pars compacta, or the median forebrain bundle (MFB)—the bundle of afferent nerve fibres projecting from the substantia nigra pars compacta to the striatum. In PD, dopaminergic loss is seen in the substantia nigra pars compacta, which results in a 70% to 80% loss of striatal dopamine by the time symptoms are visible. The MFB is the most widely used site for injection, wherein owing to anterograde and retrograde transport, the toxin degenerates both nigral and striatal dopaminergic neurons. Furthermore, cognitive deficits such as spatial memory deficit and mnemonic memory impairment are seen with the MFB model. Thus, the MFB model is a holistic representative of PD and a commonly used model.

## Methods

### Stereotactic injection of 6-OHDA in an animal model

Male Sprague-Dawley rats aged 4 to 6 weeks weighing 200 g were purchased from the Laboratory Animal Unit at The University of Hong Kong. All experimental procedures were in accordance with the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong. Animals of same treatment groups were held in pairs in cages, in a temperature-controlled room with 12 hours dark/light cycles and access to food and water ad-libitum. OXY and RES were dissolved in deionised water to make stock solutions of 1 mg/mL. Rats in the sham or 6-OHDA groups received 1, 10, or 20 mg/kg OXY, 1 or 10 mg/kg RES, or vehicle treatment. Fresh 1 mg/mL stocks of OXY and RES were prepared in water every day, and vortexed until dissolved. Drug was administered by oral gavage at the same time (12:00-14:00) every day. Rats were randomly divided into sham or 6-OHDA group, and received OXY, RES, or vehicle treatment for 1 week before unilateral stereotactic injection of 6-OHDA to induce parkinsonism (on day 8, shortly after oral gavage of drugs on that day). Fresh stock solution (3 µg/µL) of 6-OHDA hydrobromide was prepared in saline (0.9% w/v NaCl) containing 0.2 mg/mL ascorbic acid. The rats were anaesthetised with 60 mg/kg pentobarbital. 12 µg (in 4 µL) of 6-OHDA or vehicle was injected into the right MFB at the rate of 1 µL/minute using a Hamilton syringe. The coordinates for injection were selected as mediolateral (-1.2, from the Bregma), anteroposterior (-4, from the Bregma), and dorsoventral (+7.5, from the dura), with the nose bar position at 4.5, based on the atlas by Paxinos and Watson. Sham animals were injected with the same volume (4 µL) of saline as vehicle. The needle was left in place for 5 minutes before retracting, and the wound was sutured. After suturing, the animals were given meloxicam (dissolved in water) for any pain for 2 days. Rats were allowed to recover, and drug treatment continued for 2 weeks post-surgery.

### Animal behavioural tests

At the end of the 3-week treatment, three behaviour tests were carried out to assess intensity of lesion and motor dysfunction as follow:

For asymmetric cylinder test, rats were placed in a transparent acrylic cylinder for 3 minutes and their behaviour recorded. Every time the rat reared, the use of its ipsilateral, contralateral or both forelimbs was counted, for a minimum of three and a maximum of ten rears. The cylinder was cleaned with ethanol between each use. Results were expressed as % trials with ipsilateral use only.

For rotarod test, rats were trained on the rotarod before the start of the entire treatment

plan so as to generate a stable baseline. On the first day of training, rats were first made to walk on the wheel at 5 rotations per minute, to acclimatise to the instrument. Each rat was given three consecutive trials, for three consecutive days. Since the rats had stable latencies to fall by the third day, the average of the three trials on this day was used as the baseline. On the test day (day 22), the rats were given three trials on the wheel again, at the same accelerating speed of 4-40 rotations per minute, over 5 minutes. The latency to fall was noted; and the average of three trials was measured. The results were then expressed as a % decrease of the latency to fall on test day compared to the baseline.

For apomorphine-induced rotation tests, rats were injected subcutaneously with 0.3 mg/kg of apomorphine hydrochloride dissolved in saline. At 5 minutes after injection, each rat was placed in a cylinder and their behaviour recorded for 30 minutes. The number of contralateral rotations in 30 minutes was measured. A contralateral rotation rate of  $\geq 4$  rotation per minute was considered as acceptable criteria for the model.

## Results

### Effects of lesions and OXY treatment on body weight

Rats in the 6-OHDA group weighed the least post-surgery, indicating the 6-OHDA unilateral lesion had an effect on body weight. The 6-OHDA + 1 mg/kg OXY rats seemed to show the best recovery in average body weight, whereas the sham + 1 mg/kg showed the highest average body weights among all groups.

### Effects of different doses of OXY on the intensity of ipsilateral lesions

At the first stage of treatment, three doses (1, 10, and 20 mg/kg) of OXY were chosen for initial screening. The rats were randomly divided into treatment groups and administered with respective doses of OXY by oral gavage every day, for 1 week before unilateral injection of 6-OHDA. Following surgery (on day 8), OXY treatment continued daily for 2 more weeks. The effects of drug treatment on the intensity of lesion were measured on day 22 using the apomorphine induced rotation test.

After apomorphine (0.3 mg/kg) administration, rats in the 6-OHDA group had a significantly higher number of contralateral rotations in 30 minutes when compared to the sham and sham + drug groups. 10 mg/kg OXY treatment significantly reduced the number of rotations, whereas 1 mg/kg OXY showed a tendency to decrease the number of rotations. The intensity of ipsilateral lesion was thus lowered by drug treatment. Interestingly, 20 mg/kg OXY was not effective in decreasing the number of rotations.

## Effects of OXY versus RES on motor function and lesions

Rigidity of the contralateral forelimb induced by unilateral injection of 6-OHDA is a hallmark symptom of PD. An inability to use the contralateral limb and a decreased latency to fall off the rotating wheel were assessed using the cylinder asymmetric test and rotarod tests, respectively. The 6-OHDA-lesioned group showed a higher tendency to use the ipsilateral limb only while rearing, and a significantly lower latency to fall off the rotating wheel, owing to motor imbalance. However, 1 or 10 mg/kg OXY significantly reversed these results. Lesioned animals fed with 1 mg/kg OXY showed a better ability to use their contralateral limbs compared with the 6-OHDA alone group. The 10 mg/kg OXY treated animals also had a higher latency to fall off the rotarod, compared with the 6-OHDA group. The RES-treated groups did not show enhanced motor function like the OXY groups. Unlike 1 or 10 mg/kg OXY, respective doses of RES were not as effective in reducing the number of contralateral rotations. Although 1 mg/kg RES did significantly reduce the percentage of ipsilateral forelimb use, none of the doses of RES was effective in reducing the latency to fall off the rotarod, or the intensity of the lesion, as seen by the apomorphine-induced rotation test.

## Discussion

In this study, we established the model validity and the safe dosage range of OXY. Lower doses of OXY ( $\leq 10$  mg/kg) were more effective in reducing the extent of 6-OHDA lesion and motor dysfunction, whereas 20 mg/kg OXY showed little or no effects. In contrast, RES has effect on reduction in motor dysfunction at higher doses in a striatal model of 6-OHDA.<sup>2,3</sup> In this study, RES was not as effective as OXY at 1 mg/kg or 10 mg/kg. The beneficial effects of RES in an MFB model have not been elucidated. Because the striatal model is less severe than the MFB model, RES might be able to reduce cell damage and loss of motor function in the striatal model. This could explain why all three doses of RES (10, 20, and 40 mg/kg) were still effective in mitigating motor dysfunction.<sup>2</sup> In summary, we showed that OXY can be used at lower doses up to a certain range. Nonetheless, large-scale studies are warranted to determine the dosage range before any clinical studies assessing the effects of OXY on PD.

Although both 1 and 10 mg/kg OXY showed improvement in motor function, 1 mg/kg OXY did not significantly increase the latency to fall off the rotarod. This dose was however beneficial in alleviating the intensity of the lesion as well as dependence on the ipsilateral forelimb. One reason for discrepancy in these two behavioural tests was body weight. The high average body weights in

1 mg/kg OXY group might interfere with their ability to stay on the rotarod. A significant drop in weight in animals bilaterally lesioned with 6-OHDA was attributed to reduced appetite and motivation.<sup>4</sup> A certain extent of these effects might also be seen in a unilateral model, affecting their appetite. A study has shown anhedonia and neurotransmitter changes leading to depression initiated by the unilateral 6-OHDA lesion of the MFB.<sup>5</sup> Anhedonia could also affect on the appetite of animals. A gradual but constant increase in weight of the 6-OHDA + 1 mg/kg OXY-treated animals may indicate that OXY treatment could also reduce psychological changes induced by 6-OHDA, which affect inclination for food.

Taken together, OXY was more potent than RES in facilitating improvement of motor function. A protective agent like OXY seems to be suitable to combat neurodegeneration. Beneficial effects of OXY against PD should be further explored in clinical studies.

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## Disclosure

The results of this research have been previously published in:

1. Shah A, Han P, Wong MY, Chang RC, Legido-Quigley C. Palmitate and stearate are increased in the plasma in a 6-OHDA model of Parkinson's disease. *Metabolites* 2019;9:31.

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