

Antioxidant properties of a novel tyrosinase inhibitor on PC12 cells injured by hydrogen peroxide oxidative stress

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Oxidative stress is a condition caused by an overload in reactive oxygen species (ROS) that can damage biomolecules as proteins, DNA and lipids. Uncontrolled generation of ROS can lead to inflammation, tissue damage and subsequent cellular apoptosis, particularly in the brain that is more sensitive to oxidative damage than other tissues (Uttara B. *et al.*, 2009). It is known that oxidative stress plays a pivotal role in the pathogenesis of many neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Fischer R. *et al.*, 2015). It has been demonstrated that tyrosinase, a key enzyme in the synthesis of melanin in skin and hair, is expressed at low levels in the human brain (Greggio E. *et al.*, 2005), especially in the *substantia nigra* and in the dopaminergic system (Asanuma N. *et al.*, 2003). Because of Tyrosinase is involved both in the formation of dopamine-quinones and other oxidizing compounds and in the production of neuromelanin that may form a sink for such radical species in neurons susceptible in Parkinson's disease, is still unclear if tyrosinase enzyme activity is harmful or beneficial to neurons. Rat pheochromocytoma PC12 cells, a model of neuronal cells (Migheli *et al.*, 2001), have been used in this study in order to evaluate the potential antioxidant properties of the novel biphenyl tyrosinase inhibitor AD838. Hydrogen peroxide (H₂O₂) 100 µM treatment has been used to induce oxidative stress on PC12 cells. The potential protective effect of AD838 has been tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) and lactate dehydrogenase (LDH) assays. Significant decrease in cell viability was detected after 24 h of H₂O₂ treatment. However, the cytotoxic effect of H₂O₂ was partially attenuated by AD838 pretreatment (30 minutes before exposure to H₂O₂). Both assays confirmed the same trend of the AD838 effect. Moreover, the percentage of sub-G1 hypodiploid cells was measured by flow cytometry: cells were treated with AD838, H₂O₂, or pretreated with AD838 30 minutes before adding H₂O₂. The cells were fixed with 70% cold ethanol and stained with PI (iodide propidium). The treatment of cells with 100 µM H₂O₂ for 24 h significantly increased the sub-G1 peak. AD838 pretreatment (5 µM) seemed to attenuate H₂O₂-induced sub-G1 cell accumulation. To better investigate the supposed antioxidant effect of AD838, the intracellular ROS level was evaluated on H₂O₂-injured or AD838-pretreated PC12. Dichlorofluorescein diacetate chemical (DCFH-DA) was used as a substrate to measure intracellular ROS. We demonstrated that H₂O₂ exposure caused a rapid increase in the intensity of DCFDA-labeled cells that is attenuated by AD838 pretreatment, indicating a lower amount of ROS in AD838 pretreated cells. Finally, we assessed the effects of H₂O₂ and AD838 treatment on intracellular glutathione (GSH) level, since it is the major defensive mechanisms against oxidative stress-induced cell damage (Halliwell B *et al.*, 1999). GSH assay showed that one of AD838 effects is to partially restore the oxidative/antioxidant balance in the cytoplasmic environment. These preliminary results suggest the antioxidant properties of AD838 and its potential protective effect on PC12 cells. On the basis of this study and the oxidizing functions of tyrosinase in the dopaminergic system, further investigations might be required in order to suggest AD838 as a prospect for adjuvant treatment in oxidative-related diseases and its potential application in Parkinson's disease.

Uttara B. *et al.* (2009). *Curr Neuropharmacol.* 7: 65-74.

Fischer R. *et al.* (2015). *Oxid Med Cell Longev.* 610813.

Greggio E *et al.* (2005). *J Neurochem.* 93(1):246-56.

Asanuma N. *et al.*, (2003) *Neurotox Res.* 5(3):165-76.

Migheli *et al.* (2001). *J Neurochem.* 73(3):1155-63.

Halliwell B *et al.* (1999). *Oxford University Press.*