Zobec *et al.*: *Intrinsic Activity*, 2022; **10**(Suppl.2):A2.5
doi:10.25006/IA.10.S2-A2.5
published online: 16 September 2022

INTRINSIC ACTIVITY

26th Scientific Symposium of the Austrian Pharmacological Society

Graz, 23–24 September 2022

MEETING ABSTRACT

A2.5

Effect of selected drug compounds on dopamine cytotoxicity on human endothelial cells and rat astrocytes

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Background: Increased dopamine levels damage neurons and other cells in the central nervous system, thus leading to the accelerated progression of neurodegenerative diseases. The presumed mechanism of dopamine neurotoxicity is its enzymatic metabolism via monoamine oxidase B (MAO-B) to reactive metabolites with corresponding oxidative stress. Our research aimed to examine the influence of dopamine on endothelial cells and astrocytes, as well as the potential protective effects of selected drug compounds to ameliorate dopamine toxicity.

Methods: Human endothelial cells and isolated astrocytes from the cortex of neonatal rats were prepared to study the effect of dopamine on their viability depending on the dopamine concentration, exposure time, and the presence of selected drugs. To study the role of dopamine metabolism, all cells were pre-incubated with inhibitors of MAO-B and catechol-*O*-methyltransferase (COMT). To assess the role of dopamine cellular uptake, we used inhibitors of the serotonin transporter (SERT) and the noradrenaline transporter (NET). To study the role of oxidative stress, we used the antioxidant quercetin. We have also measured oxidative stress using the CAA method. To study the impact of dopamine and selected drugs on mitochondrial function, we used the Seahorse Cell Mito Stress Test kit from Agilent.

Results: Dopamine-induced time- (2–48 hours) and concentration-dependent (1 nM – 1 mM) cytotoxicity was assessed on both cell lines. Inhibition of dopamine metabolism by several MAO-B and COMT inhibitors did not increase cell viability. Similarly, inhibition of dopamine uptake by SERT and NET inhibitors had no effect. However, the reduction of oxidative stress by quercetin increased cell viability. We confirmed by the CAA method that dopamine increased cellular oxidative stress and that neither MAO-B, COMT, SERT and NET inhibitors ameliorated this condition. However, quercetin decreased oxidative stress in both cell lines. Likewise, the Mito Stress Test assay showed altered mitochondrial function due to dopamine, and no mitochondrial protection offered by inhibiting its metabolism or cellular uptake.

Discussion: Our results show that dopamine cytotoxicity is concentration- and time-dependent. Inhibition of MAO-B and COMT did not change the cell viability. This observation suggests that the mechanism of cell damage does not originate predominantly from dopamine metabolism but rather from its non-enzymatic autoxidation. We speculate that dopamine autoxidation products lead to increased oxidative stress that was responsible for observed cellular and mitochondrial toxicity. Indeed, the protective effect of quercetin confirmed this hypothesis.

Acknowledgements: This work was financially supported by the Slovenian Research Agency (research program P3-0067: Pharmacology and Pharmacogenomics).

Keywords: cytotoxicity – dopamine toxicity – mitochondrial function – oxidative stress

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