

P02.578 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS
POTENTIATE THE 1-METHYL-4-PHENYLPYRIDINIUM-
INDUCED CELL DEATH IN PC12 CELLS

N. Morioka¹, K. Kumagai¹, K. Morita¹, S. Kitayama², T. Dohi¹. ¹Hiroshima University Graduate School of Biomedical Sciences, Japan; ²Okayama University Graduate School of Medicine and Dentistry, Japan

Statement of the study: 1-Methyl-4-phenylpyridinium (MPP⁺) is known as a neurotoxin that causes the selective death of dopaminergic neuron in vivo and in vitro and widely used for experimental model of Parkinson's disease. Recently, it has been demonstrated that non-steroidal anti-inflammatory drugs (NSAIDs) have various pharmacological effects in addition to the inhibition of cyclooxygenase (COX). However, the involvement of NSAIDs in the cell death induced by neurotoxins is still unclear. In this study, we investigated the effects of different classes of NSAIDs on the MPP⁺-induced cell death in rat pheochromocytoma PC12 cells.

Methods: Cell viability was assessed by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1) assay. Accumulation of intracellular MPP⁺ or efflux of MPP⁺ from cells were measured by [³H]-MPP⁺.

Summary of results: Treatment of PC12 cells with MPP⁺ caused a concentration-dependent increase of cell death. Co-incubation of PC12 cells with 100 μ M of indomethacin, ibuprofen, ketoprofen or diclofenac, but not 1 mM of aspirin or 30 μ M of NS-398, significantly potentiated the MPP⁺-induced cell death. However, these NSAIDs had no effect on rotenone-induced cell death. Treatment with radical scavenger, antioxidant, caspase-3 inhibitor or peroxisome proliferator-activated receptor-antagonist had no influence on the stimulatory effects of these NSAIDs. Moreover, we confirmed DNA fragmentation which is one of the hallmarks of apoptosis was not induced by co-incubation with MPP⁺ and NSAIDs. We found that co-incubation of PC12 cells with 30 μ M MPP⁺ and 100 μ M indomethacin, ibuprofen, ketoprofen or diclofenac led to a significant increase in the accumulation of intracellular MPP⁺ compared with MPP⁺ alone. Furthermore, these NSAIDs remarkably reduced the efflux of preloaded [³H]MPP⁺. MK 571, an inhibitor of multidrug resistance protein 4 (MRP4), mimicked the NSAIDs-induced effects, such as increase in cell toxicity, promotion of MPP⁺ accumulation and suppression of the MPP⁺ efflux. Moreover, MRP4 mRNA was detected in PC12 cells.

Conclusion: These results suggest that NSAIDs might first block the activity of MRP4 and inhibit the efflux of MPP⁺ from PC12 cells, resulting in the retention of cytosolic MPP⁺, which consequently promotes cell toxicity.