Paraquat and maneb differently affect opioid receptor gene expression in human neuroblastoma SH-SY5Y cells

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Parkinson's disease (PD) is a neurodegenerative disorder mainly affecting the dopaminergic cells of the substantia nigra pars compacta. Neuronal toxicity involves the aggregation of alpha-synuclein leading to the formation of intraneuronal cytoplasmic inclusions termed "Lewy Bodies".
The exposure to environmental factors, such as pesticides, has been suggested to increase the risk for the development of PD (Costello et al., 2009). Paraquat (PQ) (1,1'-dimethyl-4,4'-bipyridinium dichloride) is a highly toxic quarternary nitrogen herbicide widely used in agriculture; the exposure to PQ induces cellular toxicity mainly by the oxidative stress and apoptotic-related mechanisms. Moreover, PQ has been shown to induce alpha-synuclein aggregation and to increase the alpha-synuclein-induced toxicity (Franco et al., 2010).
Maneb (MB) is Mn-containing ethylene-bis-dithiocarbamate fungicide widely used in the world; the exposure to MB has been also demonstrated to induce oxidative stress, alpha-synuclein aggregation, mitochondria alteration, and to be related to the risk of PD (Franco et al., 2010).
In addition, *in vivo* studies demonstrated that the simultaneous exposure to PQ and MB induces synergistic toxic effects on nigrostriatal dopaminergic neurons.
The first aim of the present study was to investigate the effect of human neuroblastoma SH-SY5Y cell exposure to PQ and MB on the Tyrosine hydroxylase (TH) and alpha-synuclein levels using Western blotting. The second aim of this investigation was to study the MOP, DOP, and NOP gene expression (by real-time PCR) in the same SH-SY5Y cell line; the opioid system is in fact known to be implicated in motor functions, and opioid receptors are involved in regulating dopamine functions.
To these purposes, cell were exposed to low and high doses of PQ and MB separately or to their association in order to investigate the effect of single pesticide and the possible synergistic effect of the association. The exposure to low and high doses has been chosen since the effect of the acute accidental exposure to high dose is likely different compared to the more frequent chronic low dose exposure as occurring in agriculture workers. The pesticide concentrations were chosen on the basis of the MTT cell viability data following 48 h of pesticide exposure. SH-SY5Y cells were then exposed to 100 μM PQ, 6 μM MB, or their association (low doses, LD), or to 250 μM PQ, 15 μM MB or their association (high doses, HD).
Results showed that the level of TH was significantly increased following cell exposure to the LD PQ and MB association; the other pesticide cell treatments triggered no significant differences of TH levels compared to control cells.
The level of alpha-synuclein was significantly increased following the LD PQ and the LD PQ and MB association, whereas it decreased after the HD PQ cell treatment.
Moreover, the cell exposure to LD and HD pesticides or their association induced selective changes of MOP, DOP, and NOP gene expression.
The present data showed selected changes of TH and alpha-synuclein levels, and of opioid receptor gene expression following PQ and MB cell exposure suggesting that this *in vitro* model is suitable to study the biochemical opioid-related mechanisms underlying the pesticide exposure. These data also contribute to define the role of molecular changes in the endogenous opioid system triggered by pesticides and their possible meanings in the environmental pathogenesis of PD.

Costello S. et al. (2009). *Am. J. Epidemiol*. 169, 919-926.
Franco R. et al. (2010). *Chem. Biol. Interact.* 188, 289-300.