Nicotine may stimulate autophagy via ROS-mediated adaptive response

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Aggregation of misfolded proteins is a hallmark of neurodegenerative brains. In late-onset Parkinson's disease (PD), pro-filamentous protein accumulation is coupled with an age-related overstrain of the ubiquitin-proteasome system. Autophagy, an alternative cellular catabolic process, can compensate the proteasome system decline. Despite the harmful effects of cigarette smoking on health, nicotine consumption has been shown to lower the risk of PD [1]. Based on the premise that nicotine at its hormetic concentration may induce autophagy, we investigated the possible effect of nicotine on autophagy and cellular lifespan in human neuronal cells.

Keywords: Aging; Autophagy; Neurodegeneration; Reactive oxygen species (ROS).

Nicotine showed hormetic effect on cellular chronological lifespan

An established chronological lifespan (CLS) assay was first performed to determine the hormetic concentration of nicotine in SH-SY5Y cells [2]. Nicotine treatment at 1 mM and 0.3 mM concentrations led to significantly extended cellular lifespan (Fig. 1).

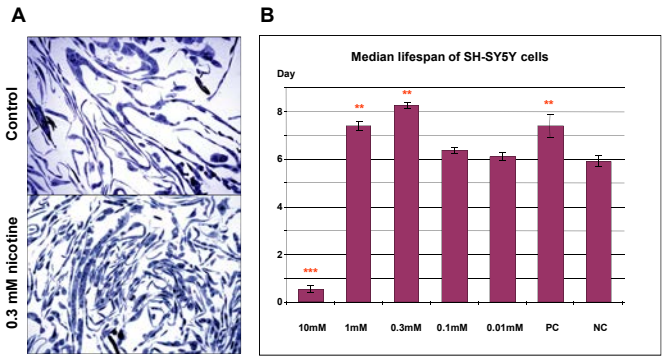

Fig.1: *Chronological lifespan (CLS) assay in SH-SY5Y cells under nicotine treatment. **A**: Healthier cell morphorlogy was observed in nicotine treated cells. **B**: Nicotine affects the median CLS of SH-SY5Y cells in a dose-dependent manner. Nicotine treatment at low dose (0.01 and 0.1 mM) showed no effect; high dose of nicotine (10 mM) led to premature cell death. In the presence of 1mM and 0.3 mM nicotine CLS was significantly increased in comparison to the control. NC: untreated cells; PC: positive control treatment with rapamycin. Asterisks indicate statistical significance compared to control. ***: p<0.001; **: p<0.01.*

Nicotine up-regulated the number of autophagy-positive cells

Making use of a green flourophore that specifically stains autophagic vacuoles (AV), we observed that 35% and 38% cells were AV-positive during the culturing period 48-72h in 1 mM and 0.3 mM nicotine treated cells, respectively. This is significantly higher than the basal autophagy level (Fig. 2).

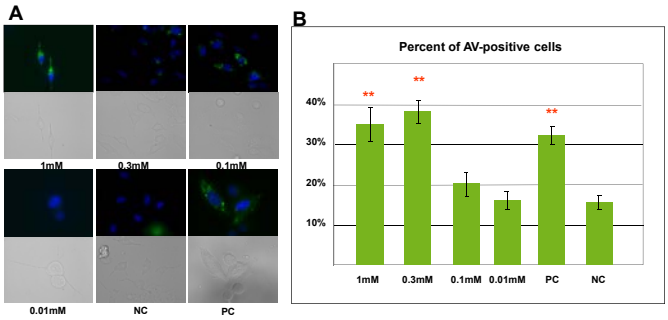

Fig.2: *Fluorescence staining of AV in nicotine treated cells. **A**: AV can be distinguished by their punta-like green fluorescence in the cytosol. **B**: Quantification of AV-positive SH-SY5Y cells 48-72 hours after nicotine treatment. Non-treated cells showed a basal autophagy level of 15.5%. Nicotine treatment at 1 mM and 0.3 mM up-regulated the level of autophagy to 35% and 38%, respectively. **: p<0.01.*

Up-regulation of mitophagy was revealed by electron microscopy

By electron microscopy (EM), we observed that the number of autophagosomes was higher in nicotine-treated cells compared to control cells. Moreover, the number of healthy mitochondria was significantly higher in nicotine-treated cells compared to control (Fig. 3).

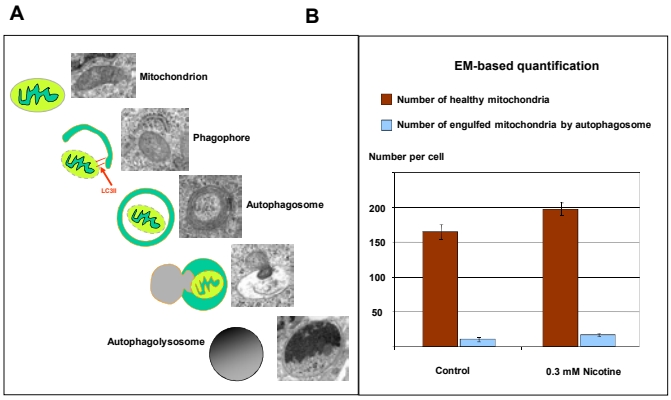

Fig.3: *Transmission electron microscopy analyses of SH-SY5Y cells after three days of nicotine treatment at 0.3 mM. **A:** Substages of mitophagy as revealed under EM. **B:** There was a significant up-regulation of mitophagy in nicotine-treated cells. Moreover, nicotine treated cells contained significantly more healthy mitochondria.*

Nicotine induced an early ROS peak

Cells under nicotine treatment were measured for their time-dependent ROS level using DHR123. The time point exerting the highest ROS-level in the cultured cell (termed "ROS-peak") was observed on day 3 in control, whereas this was one day earlier in all nicotine-treated cells (Fig. 4).

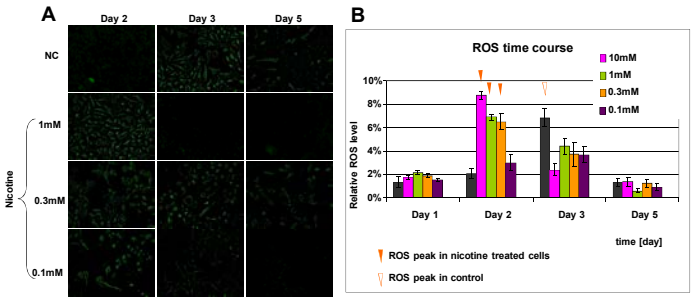

Fig.4: *Image-based intracellular ROS quantification after staining with DHR123 on day 2, 3 and 5 of nicotine treatment. **A**: Nicotine-treated cells showed significantly higher ROS level on day 2. In contrast, control cells reached its ROS peak at day 3. **B**: Time course of ROS level in cultured SH-SY5Y cells under nicotine treatment at different concentrations. Percent of ROS fluorescence area were normalized with total area of cells on each micrograph.*

Discussion: Treatment of nicotine in human neuronal cells at hormetic concentrations can prolong cellular chronological lifespan. More autophagy incidences were observed via different approaches in the presence of nicotine. Intracellular measurements of ROS suggest that such positive effect of nicotine could be mediated by an early peak of reactive oxygen species (ROS), which triggers cellular adaptive responses [4]. Our preliminary results support the idea that stimulation of autophagy by nicotine-related drugs may help to degrade misfolded proteins and defective organelles and thus act against neurodegeneration.

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Literatures:

[1] Chen, H., et al., Neurology, 2010.
[2] Leontieva, O.V. & M.V. Blagosklonny. Aging (Albany NY), 2011.
[3] Klionsky, D.J., et al., Autophagy, 2012.
[4] Schmeisser, S., et al., Mol Metab, 2013.

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