Inhibition of JNK provides neuroprotection against 6-OHDA toxicity in Parkinson's disease in vitro model

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INTRODUCTION

- **Parkinson's disease (PD) is a neurodegenerative disorder marked by death of dopaminergic neurons.**
- **PD is caused by oxidative stress, which may be induced by pro-oxidants like 6-OHDA.**
- **JNK is a major pro-apoptotic kinase, which plays a key role in dopaminergic degeneration.**

OBJECTIVES

The present study aimed to investigate the effect of pharmacological JNK inhibition in cellular model for PD.

METHODS

1. SH-SY5Y cells were differentiated with 10 μM retinoic acid (RA) for 7 days prior to experiments.
2. Neurodegeneration was induced by treatment with 6-OHDA at EC50. Cells were treated with JNK inhibitor V either 1 h before or after 6-OHDA-induced damage.
3. The effect of JNK V on cell viability upon 6-OHDA toxicity was measured by XTT assay.
4. The genotoxicity was assessed by the comet assay.
5. The mRNA expression level of specific pro-apoptotic ER stress-related genes was measured by RT-qPCR.

RESULTS

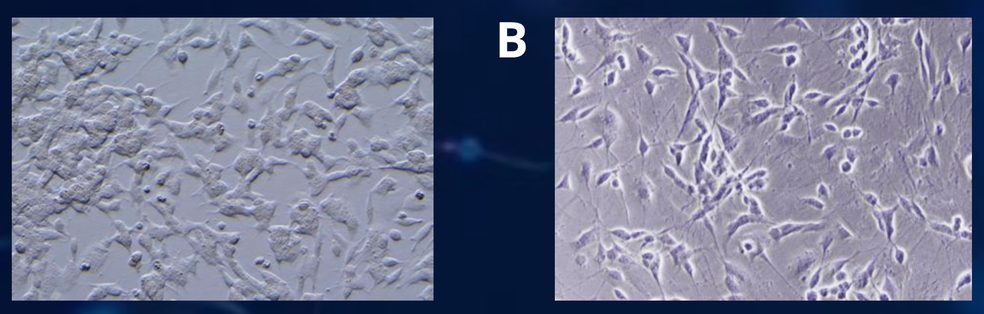

Fig. 1. Morphological features of undifferentiated SH-SY5Y cells (**A**) and SH-SY5Y cells differentiated with RA (**B**). Undifferentiated cells are characterized by short processes and tend to grow in clusters, whereas differentiated cells have significantly longer neurites and communicate with each other via neural network.

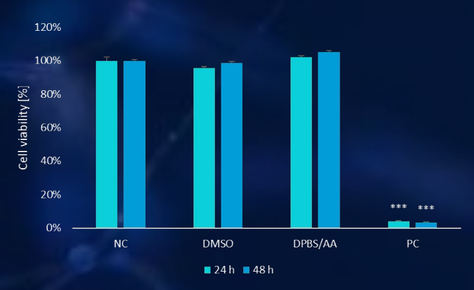

Fig. 2. Cytotoxicity analysis of the used compounds' solvents in differentiated SH-SY5Y cells using XTT assay. The data are presented as mean ± SD. ***p<0.001 vs. NC. NC – negative control, untreated cells; DMSO – 0.1% DMSO; DPBS/AA – 0.5% DPBS with 0.15% w/v ascorbic acid, PC – positive control, cells treated with 20% DMSO.

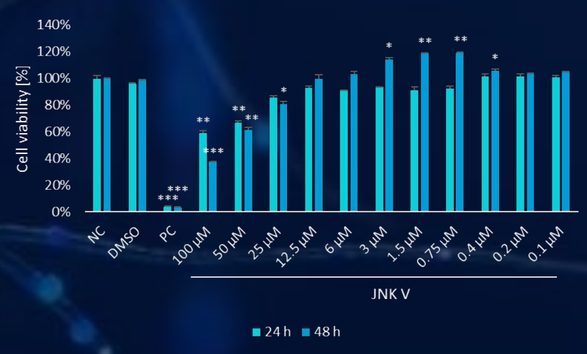

Fig. 3. Cytotoxicity analysis of JNK V inhibitor in differentiated SH-SY5Y cells using XTT assay. The data are presented as mean ± SD. *p<0.05, **p<0.01, and ***p<0.001 vs. NC. NC – negative control, untreated cells; DMSO – 0.1% DMSO; PC – positive control, cells treated with 20% DMSO.

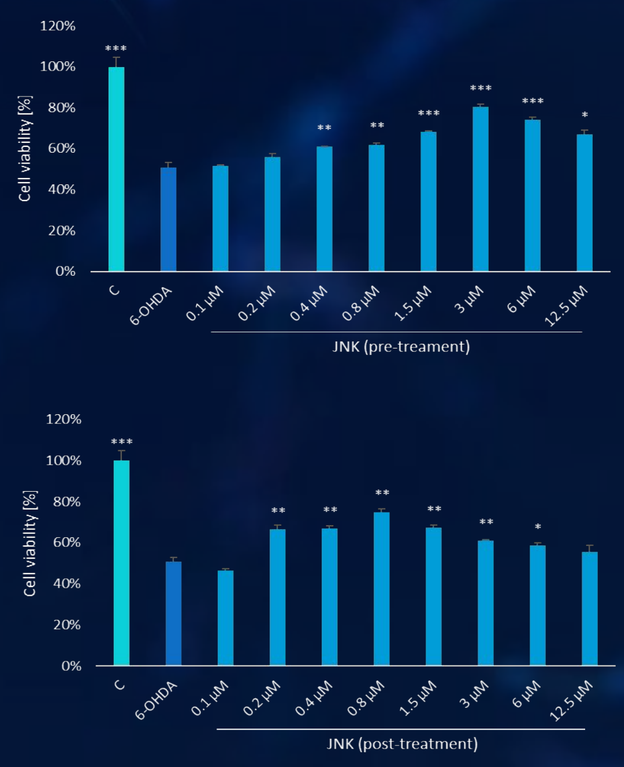

Fig. 4. The effect of pre-treatment with JNK V on the viability of differentiated SH-SY5Y cells exposed to neurotoxin 6-OHDA. The data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001 vs 6-OHDA. C – control, untreated cells; JNK – JNK V.

Fig. 5. The effect of post-treatment with JNK V on the viability of differentiated SH-SY5Y cells exposed to neurotoxin 6-OHDA. The data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001 vs 6-OHDA. C – control, untreated cells; JNK – JNK V.

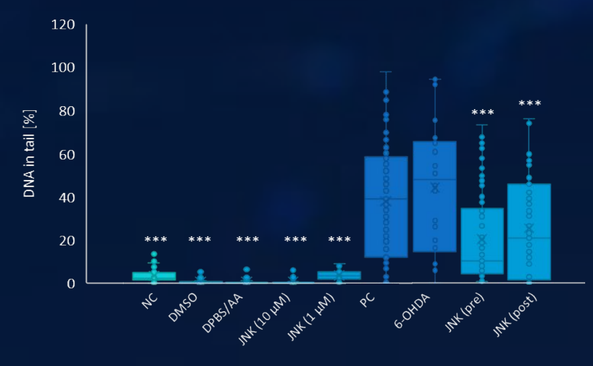

Fig. 6. Genotoxicity of the solvents and JNK V inhibitor in differentiated SH-SY5Y cells, and the effect of treatment with JNK V on the level of DNA damage in differentiated SH-SY5Y cells exposed to 6-OHDA. ***p<0.001 vs 6-OHDA. NC – negative control, untreated cells; DMSO – 0.1% DMSO; DPBS/AA – 0.5% DPBS with 0.15% ascorbic acid, PC – positive control, cells treated with 20% DMSO; JNK - JNK V.

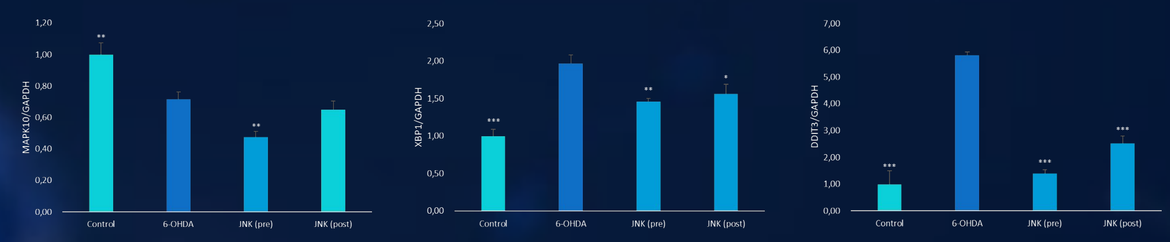

Fig. 7. The mRNA expression levels of *MAPK10*, *XBP1* and *DDIT3* genes in differentiated SH-SY5Y cells exposed to 6-OHDA and treated with JNK V inhibitor. *GAPDH* was used as a reference gene. The data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001 vs 6-OHDA. C – control, untreated cells; JNK – JNK V.

CONCLUSIONS

Small-molecule JNK V inhibitor is effective against 6-OHDA-induced damage *in vitro*. Thus, JNK inhibitors could potentially be applied for the selective treatment of PD.

This work was supported by grant PRELUDIUM BIS 3 no. 2021/43/O/NZ5/02068 from the National Science Centre, Poland.

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