NEUROPROTECTIVE THERAPEUTIC STRATEGY AGAINST NEURONAL DAMAGE

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Background/Purpose

Neurodegenerative diseases are one of the greatest challenges for science and clinical medicine due to their complex pathology and the lack of treatment methods based on mechanisms [1]. Emerging neuronal dysfunction is associated with cellular and molecular changes that trigger a cascade of events and, consequently, the death of nerve cells. The changes involved in neurodegeneration include an increased level of oxidative stress, impairment of mitochondrial function, activation of apoptotic factors, an abnormal mechanism of the cell cycle as well as an increased level of cellular calcium and DNA damage. However, an effective drug therapy for treating neurodegenerative diseases has not yet been developed [2]. Therefore, the aim of this research is to develop an new neuroprotective method in the treatment of these diseases.

Methods

For all expermients, the mouse hippocampal neuronal cells (HT-22) and neuroblastoma cell line (SH-SY5Y) were used. Normally, cells were used grown and maintained at 37°C, 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) and Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F12), supplemented with 10% FBS and antibiotic mix solution. Compound D2AAK1 was used for the research [3].

Cellular oxidatve stress parameters: The superoxide, nitric oxide and reduced glutathione levels were evaluated using DHE, DAF-FM diacetate and Thiol Tracker respectively, as described in manufacturer's protocols. Digital images were captured with an InCell Analyzer 2000. Quantitative analysis was conducted using ImageJ software and presented as relative fluorescence units (RFU).

Micronuclei (MN) detection: Nuclei were visualized with Hoechst 33258. Digital Images were captured with InCell Analyzer 2000 and results are presented as % of positive cells of total cells from minimum of 1000 cells.

Cell cycle analysis: The cell cycle profile was evaluated using DNA Cell cycle plug-in from ImageJ software. Results are presented as % of cells in each of G0/G1, S and G2/M phases.

Statistical analysis: All experiments were conducted at least in triplicate The statistical analysis of the results was performed using GraphPad Prism ver. 8.0. Data shown represent the means ± standard deviation. Differences between control and test samples were assessed with one-way analysis of variance with Dunnett post hoc test. A p-value of <0.05 was considered as statistically significant between groups and are displayed as:
*, p<0.05; **, p <0.01; ***, p<0.001.

Results

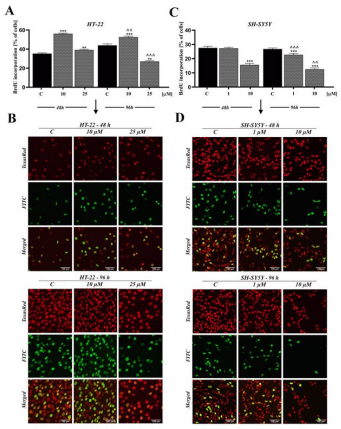


Fig. 1. The effect of D2AAK1 on HT-22 cells (A,B) and SH-SY5Y cells (C,D) in BrdU assay.

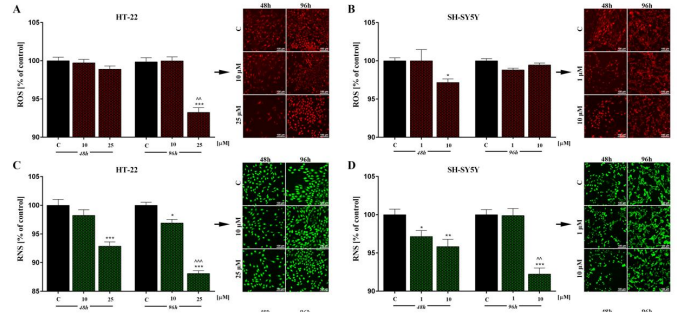
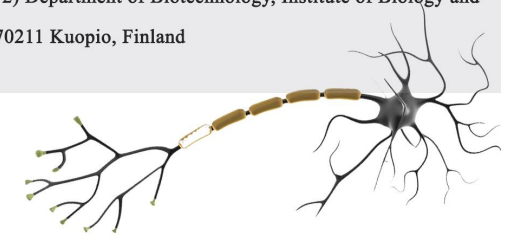


Fig.2 . D2AAK1-mediated effects on HT-22 and SH-SY5Y cells in terms of ROS and RNS (A-D).



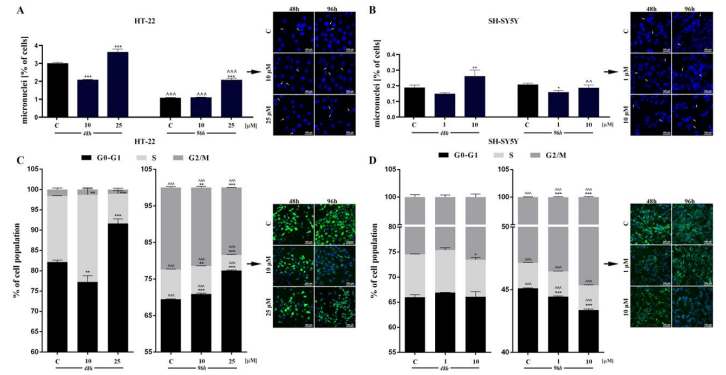


Fig.3. D2AAK1-mediated effects on HT-22 and SH-SY5Y cells in terms of micronuclei formation (A-B) and cell cycle profile (C-D).

Conclusions

- The compound increases the proliferation of hippocampal neurons.
- The compound has antioxidant properties, reducing the level of free radicals.
- The compound at a concentration of 10 µM reduces the level of DNA damage in the form of micronuclei.
- The compound has a neuroprotective effect

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