Protection against hydrogen peroxide-induced injury in PC12 cells by 2-methoxy-6-acetyl-7-methyljuglone isolated from ***Polygonum cuspidatum***

Speciality: Traditional Chinese pharmacology

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Abstract

Objective

Neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), are defined by the progressive loss of specific neuronal cell populations and are associated with protein aggregates. Because of its high metabolic rate and relatively reduced capacity for cellular regeneration compared with other organs, the brain is believed to be particularly susceptible to the damaging affects of ROS.

The treatment for neurodegenerative diseases is not very good.The Traditional Chinese medicine has its speciality. Exploring the effective Traditional Chinese medicine is meaningful.

The purpose of the present study is to determine the protective of 2-methoxy-6-acetyl -7-methyljuglone of *Polygonum cuspidatum*, to assess its possible protective effects on neurons from oxidative stress, as a preliminary step in the understanding of its mechanism of action.

Methods

In this study, cell viability assay, morphological assay, flow cytometry detection of cell death and mitochondrial membrane potential assay were used to investigate whether 2-methoxy-6-acetyl-7-methyljuglone exerted neuroprotective effects on H_2O_2 induced injury in PC12 cells and to study the possible mechanisms.

1. Establish H_2O_2 induced PC12 cell injury model.

2.Apply MTT asssy to detect the cell viability of different concentrations of ethyl acetate

extract(EAPC), petroleum ether extract (PAPC), 95% ethanol extract (EEPC) of *Polygonum cuspidatum* on PC12.

3. The effect of the EAPC and the compounds isolated from EAPC on H_2O_2 induced PC12 injury by LDH, MTT assay.

4. Effect of 2-Methoxy-6-acetyl-7-methyljuglone on H_2O_2 induced changes in mitochondrial membrane potential.

5. Adopt Annxin V/PI assay to detect the apoptosis and necrosis rate of e2-Methoxy-6-acetyl-7-methyljuglone on on H_2O_2 -induced injury.

6. Nuclear staining for assessment of apoptosis was observed using the chromatin dye Hoechst 33324.

7. Effect of 2-Methoxy-6-acetyl-7-methyljuglone on the cell cycle by H_2O_2 induced injury in PC12 cells.

Results

1.880μM H_2O_2 induced PC12 injury model was estabilshed .

2.The results showed that the concentration of MAM from 0.3μM to 9.6μM has no cytotoxicity.

3. Following exposure of the cells to 880μM H_2O_2 for 24h, the significant reduction in cell survival and activities lactate dehydrogenase (LDH) release.The cell survival were increased and the LDH release is reduced by MAM (0.3-9.6μM) plus 880μM H_2O_2.

4.MAM(4. 8μM、1. 2μM、0. 3μM) can reduce 880μM H_2O_2-induced mitochondrial membrane potential change of JC-1 stained mitochondria in PC12 cells at 2H.

5.The apoptotic nature of H_2O_2-induced cell death was further confirmed by annexin V-FITC labeling of PS exposed on the plasma membrane. MAM(4. 8μM、1. 2μM、0. 3μM) can inhibit the apoptosis .

6.Further evidence for the apoptotic nature of the observed cell death was provided by the nuclear morphology by using Hoechst 33324 under a microscope. H_2O_2 clearly induced nuclear fragmentation. MAM(4.8μM、1.2μM、0.3μM) can reduce the nuclear fragmentation.

7.Since apoptosis and the cell cycle are closely linked, EAPC changed the cell cycle exposure to H_2O_2 for 24h. MAM(4.8μM、1.2μM、0.3μM) inhibit the S stage.

8.The EAPC and the compounds from EAPC by MTT, LDH assay. The results showed that EAPC, polydatin, Anthraglycoside A and Anthraglycoside B have protective effects.

Conclusion

The concentrations of MAM at 4. 8μM、1. 2μM、0. 3μM can reduce the release of LDH, increase the depolarization of mitochondrial membrane potential (MPP), reduce the apoptosis rate and nuclear fragmentation. Taken together, these results suggest that MAM shows protection against H_2O_2-induced cell injury.

Keywords

***Polygonum cuspidatum*; 2-methoxy-6-acetyl-7-methyljuglone; hydrogen peroxide; PC12 cells; protection**