

PROTECTIVE EFFECT OF GENISTEIN-LOADED TRANSFEROSOMES AGAINST H₂O₂-INDUCED OXIDATIVE STRESS IN PC12 CELLS

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Oxidative stress is a condition determined by the excessive amount of reactive oxygen species (ROS) that can damage biomolecules as proteins, lipids and nucleic acids and consequently results in many cellular dysfunctions. The ROS overproduction has been regarded as an important factor characterizing different neurodegenerative disorders including Parkinson's and Alzheimer's diseases (Fischer R. et al., 2015). Rat pheochromocytoma PC12 cells, a model of neuronal cells (Migheli R. et al., 2001), have been used in this study to evaluate the potential antioxidant properties of Genistein (GEN), the predominant component in soy products, known in literature as Tyrosine kinase and Topoisomerase II protein inhibitor, and already tested in anticancer therapy (Weiwei M. et al., 2010). Four formulations of Genistein-loaded transferosomes (GEN-TFs) were used as drug delivery systems in PC12 cells exposed to oxidative stress induced by hydrogen peroxide (H₂O₂ 75 µM). The effect of GEN-TF1, -TF2, -TF3, -TF4 (30 µM) was assessed, after 24h hydrogen peroxide exposure, using MTT reduction assay (Maioli M. et al., 2015). The cytotoxic effect of H₂O₂ was particularly attenuated by GEN-TF2 pretreatment (30 minutes before hydrogen peroxide exposure). Further investigations were effectuated to underline the GEN-TF2 antioxidant effect by means of LDH cytotoxicity assay (Meizhu Z. et al., 2016) confirming its protective activity against the oxidative damage. Moreover, the intracellular ROS levels were measured with Dichlorofluorescein-diacetate (DCFH-DA) both in PC12 cells H₂O₂-injured and GEN-TF2 pretreated. GEN-TF2 was able to reduce amount of ROS in labeled cells exposed to oxidative damage. In parallel, the nuclear integrity of PC12 cells it was assessed by fluorescence microscopy, after Hoechst 33342 staining, highlighting a reduction of the apoptotic nuclei in presence of GEN-TF2 and hydrogen peroxide. In addition, the Genistein intracellular uptake by PC12 cells was evaluated and confirmed by flow cytometry using the fluorescent GENT-TF2 formulation. Detailed studies were effectuated to investigate on cell death mechanism. The percentage of sub-G1 hypodiploid cells was assessed by PI (propidium iodide) staining in PC12 cells treated with H₂O₂ or pretreated with GEN-TF2 and H₂O₂ for 24h. A considerable decrease of the percentage of sub-G1 peak was shown in presence of GEN-TF2 and H₂O₂ compared to the sub-G1 peak induced by hydrogen peroxide alone. Moreover, cell apoptosis was assessed using AnnexinV/7-AAD staining under the same experimental conditions (Pinna A. et al., 2015). The apoptotic cell percentage was significantly reduced in the samples pretreated with GEN-TF2, confirming its protective effect. In conclusion, these obtained data assign to GEN-TF2, drug delivery system, a potential antioxidant activity and its possible application as adjuvant therapy in oxidative stress-related neurodegenerative diseases, such as Parkinson.

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