Abstract

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Title of diploma thesis: Modulation of anti-inflammatory response in microglial cell line SIM-A9

In the theoretical section, the thesis is focused on the characteristics, significance, and contribution of neuroinflammation in the origin and development of neurodegenerative diseases. The possibilities of their current treatment are discussed. The experimental part is devoted to evaluating the cytotoxicity and anti-inflammatory response of newly synthesized derivatives of naphthoquinones (K1502, K1503 and K1504) and chromones (5-EM-13, 5-EM-26 and 5-EM-36). Cytotoxicity was measured in the mouse microglial line SIM-A9. In addition, the neuroblastoma line SH-SY5Y was used comparatively in this part. The half-maximal inhibitory *concentration (IC_{50})* and maximum tolerated concentration (MTC) were determined on both cell lines using MTT colorimetric methods. The safety of MTC was subsequently verified by microcapillary flow cytometry in the SIM-A9 cells after lipopolysaccharide (LPS) or interferon-γ (IFN-γ) stimulation. The anti-inflammatory effects of the studied substances were further monitored in SIM-A9 cells. The substances were applied in concentrations corresponding to 100%, 50%, and 25% MTC. Anti-inflammatory effects were measured using two methods. The first method, enzyme-linked immunosorbent assay (ELISA), was used to determine concentrations of proinflammatory cytokines IL-6 and TNF-α. LPS was added 1 hour after the application of substances. Cytokine concentrations were subsequently measured 23 hours after LPS administration. TNF-α production was assessed in all studied substances, while IL-6 levels were determined only in naphthoquinone derivatives. As a second method, the Griess reaction was utilized to determine nitric oxide (NO) levels produced by the cells into the culture medium after IFN-γ stimulation. IFN-γ was added to SIM-A9 cells 1 hour after drug application. NO levels were determined 47 hours after IFN-γ administration in all test substances. The results of the thesis show that naphthoquinone derivatives display higher

cytotoxicity than chromone compounds. The most toxic substance was K1504. Modulation of the inflammatory response induced by LPS or IFN-γ was affected by only one substance K1503. This naphthoquinone significantly increased TNF-α secretion at all studied concentrations in the presence of LPS. On the other hand, K1503 did not affect IL-6 production. After stimulation with IFN-γ, K1503 inhibited NO production by 27% at the highest tested concentration, corresponding to MTC. The results did not prove any anti-inflammatory effects of new naphthoquinone and chromone derivatives. However, naphthoquinone K1503 could serve as a starting structure in searching for other biologically active substances.

Keywords: neuroinflammation, neuroglia, neurodegenerative diseases, naphtoquinone derivatives, chromone derivates