Title: MCCP induces pro-apoptotic endoplasmic reticulum stress is mediated by GSK3α/β activation in human colon cancer cells HCT-116

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Aims:. Rupture of the colon tumor may become a surgical emergency. Scientists worldwide have been interested to search novel anticancer agents. The new natural phloroglucinol derivatives (3, 6-bis (3-chlorophenylacetyl)phloroglucinol, MCPP) have been identified as possessing anticancer activity. Endoplasmic reticulum stress (ER) might triggers several specific signaling pathways and plays a crucial role in the regulation of apoptosis. The aim of this study is to investigate the influence of the MMCP on the pro-apoptotic endoplasmic reticulum stress in colon cancer (HTC-116 cells).

Methods: The human colon carcinoma cell lines HCT-116 were obtained from the American Type Culture Collection (Manassas, VA). The percentage of apoptotic cells was analyzed by flow cytometry of Annexin V/PI double staining. Total RNA was extracted from cells using a TRIzol kit (MDBio, Inc.,Taipei, Taiwan). The reverse transcription reaction was performed using 2 mg of total RNA that was reverse transcribed into cDNA. The western blots analysis was visualized by enhanced chemiluminescence using Kodak X-OMAT LS film (Eastman Kodak, Rochester, NY). The difference was significant if $p < 0.05$. (ANOVA).

Results: MCPP induced eIF2α phosphorylation in a time-dependent manner. Treatment of HCT-116 cells with eIF2α inhibitor did not reduce MCPP-mediated cell apoptosis. GSK3β is a kinase that plays an important role in the regulation of cell survival. MCPP reduced phosphorylation of GSK3α/β at Ser21/9, whereas it stimulated phosphorylation of GSK3α/β at Tyr270/216 in HCT-116 cells. MCPP had little effect on the expression of GSK3α/β. Treatment of cells with GSK3 inhibitor SB216763 reduced MCPP-mediated cell apoptosis. Treatment of GSK3 inhibitor SB216763 also dramatically reversed MCPP-induced GRP and CHOP up-regulation, and pro-caspase-3 and pro-caspase-9 degradation. Pretreatment of SB216763 also reduced caspase-7 cleaved form expression.

Conclusions: We synthesized the new phloroglucinol derivative MCPP and investigated its anticancer activity in human colon cancer cells. Taken together, our results suggest that GSK3α/β activation but not eIF2α is involved in MCPP-mediated HCT-116 human colon cancer cell deaths, and also strongly support the proposed beneficial effects of MCCP as the molecular-targeted therapy in the anti-tumorigenesis.

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