Caffeine inhibits the growth of glioblastomas through activating the caspase-3 signaling pathway in vitro.

Liu JD, Song LJ, Yan DJ, Feng YY, Zang YG, Yang Y

Abstract

OBJECTIVE: To study the effects and associated mechanisms of caffeine on cell viability, cycle dynamics, proliferation and apoptosis both in glioblastoma C6 and U87MG cells.

MATERIALS AND METHODS: Cell livability in presence or absence of caffeine was detected by the methyl thiazolyl tetrazolium (MTT) colorimetric assay. Flow cytometric analysis was conducted to investigate the cell cycle dynamics and Cell Counting Kit-8 (CCK-8) was used to further study the proliferation of C6 and U87MG glioblastoma cells after treated with caffeine or DMSO. To study the influence of caffeine on apoptosis of glioblastoma C6 and U87MG cells, the value of apoptosis ratio (AR) was calculated by flow cytometry detection. Western blot analysis was used to detect the expression of apoptosis-related factors, including Caspase-3, Cyt-C, Bax and Bcl-2.

RESULTS: Caffeine at 1 mM reduced the cell viability of the both rat C6 and human U87MG glioblastoma cells to less than 70%. Flow cytometry detection found that caffeine remarkably arrested the C6 and U87MG cells in G0/G1 phase (C6, U87MG: p<0.01, p<0.05). Nevertheless, the percentage of cells in S phase obviously decreased in the caffeine-treated group, when comparing to that of the normal control (C6, U87MG: p<0.01, p<0.01). CCK-8 assay demonstrated that significant decreases in the number of glioblastoma cells were observed in caffeine treatment group, when comparing to that of the normal control (C6, U87MG: p<0.01, p<0.05). Flow cytometric analysis also found that the application of caffeine induced much higher apoptosis of glioblastoma cells, compared with the normal control (C6, U87MG: p<0.01, p<0.05). Furthermore, caffeine markedly reduced the expression of Bcl-2 (C6, U87MG: p<0.01, p<0.01), and promoted the expression of Cyt-C (C6, U87MG: p<0.05, p<0.01) and Caspase-3 (C6, U87MG: p<0.01, p<0.01), comparing to the normal control.

CONCLUSIONS: Caffeine inhibits proliferation and induces apoptosis of C6 and U87MG cells, leading to an imbalance in the ratio of proliferation and apoptosis. The apoptosis might be promoted by the motivation of the caspase-3 signaling pathway, which is induced by the release of Cyt-C as well as the elevated rate of Bax/Bcl-2.