Mulberry fruit (Moris fructus) extracts induce human glioma cell death in vitro through ROS-dependent mitochondrial pathway and inhibits glioma tumor growth in vivo.

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Abstract
Mulberry has been reported to contain wide range of polyphenols and have chemopreventive activity. However, little has been known regarding the effect of mulberry fruit extract on cell viability in vitro in human glioma cells and the anticancer efficacy in vivo. This study was undertaken to examine the effect of mulberry fruit (Moris fructus; MF) extracts on cell viability in vitro and anticancer efficacy in vivo. Cell viability and cell death were estimated by MTT assay and trypan blue exclusion assay, respectively. Reactive oxygen species (ROS) generation was measured using the fluorescence probe DCFH-DA. The mitochondrial transmembrane potential was measured with DiOC(6)(3). Bax expression and cytochrome c release were measured by Western blot analysis. Caspase activity was estimated using colorimetric kit. Cell migration was estimated using the scratched wound model. In vivo anticancer efficacy of MF extracts was evaluated using a subcutaneously injected mouse tumor model. Changes in proliferation and apoptosis were estimated by immunohistochemical analysis. MF extracts resulted in apoptotic cell death in a dose- and time-dependent manner. MF extracts increased ROS generation, and the MF extract-induced cell death was also prevented by antioxidants, suggesting that ROS generation plays a critical role in the MF extract-induced cell death. Western blot analysis showed that treatment of MF extracts caused an increase in Bax expression, which was inhibited by the antioxidant N-acetylcysteine (NAC). MF extracts induced depolarization of mitochondrial membrane potential, and its effect was inhibited by the antioxidants NAC and catalase. MF extracts induced cytochrome c release, which was inhibited by NAC. Caspase activity was stimulated by MF extracts, and caspase inhibitors prevented the MF extract-induced cell death. Treatment of MF extracts inhibited cell migration. Oral MF extracts administration in animals with subcutaneous U87MG glioma cells reduced tumor volume. Subsequent tumor tissue analysis showed a decrease in PCNA-positive cells, an increase in TUNEL-positive cells, and caspase activation. From these data, we concluded that MF extracts reduce glioma tumor growth through inhibition of cell proliferation resulting from induction of apoptosis. These findings suggest that MF extracts result in human glioma cell death in vitro through ROS-dependent mitochondrial pathway and glioma tumor growth in vivo via reduction of tumor cell proliferation and induction of apoptosis.

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