Effects of valproic acid on the susceptibility of human glioma stem cells for TMZ and ACNU.


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To investigate the effect of valproic acid (VPA) on the susceptibility of glioma stem cells to temozolomide (TMZ) and nimustine (ACNU), the O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation and its expression of MGMT were examined. A total of 3 glioma cell populations were isolated from human glioma tissues, and immunocytochemistry was used to detect the expression of MGMT. VPA inhibition on the growth of the 3 glioma cell populations exposed to various concentrations of TMZ and ACNU was evaluated. Flow cytometry was applied to detect the apoptosis of glioma cells, and a methylation-specific polymerase chain reaction was used to identify methylation of MGMT promoter. Immunocytochemistry results indicated that MGMT was negatively expressed in the G1 population, but positively expressed in the G2 and G3 populations. Cell growth inhibition assays demonstrated that the survival rate in the VPA + TMZ or ACNU groups was decreased compared with that of the TMZ or ACNU alone groups (P<0.05). As for the apoptotic rate, those in the VPA alone group were increased compared with the control group (P<0.05), and the rates in the VPA + TMZ or ACNU groups were increased compared with TMZ or ACNU alone groups (P<0.05). The expression of MGMT remained negative in the G1 population following treatment with VPA, but MGMT expression became negative in the 2 MGMT-positive cell populations (G2 and G3) following VPA treatment. The MGMT promoter in the G1 population was partially methylated in the control group, but was fully methylated following VPA treatment, while the promoters of G2, G3 were unmethylated in the control group and became partially methylated in the VPA treatment group. Taken together, TMZ and ACNU may suppress the growth of glioma stem cells in vitro in a dose-dependent manner. VPA may enhance the inhibitory effects of various concentrations of TMZ and ACNU on the growth of MGMT-negative/positive cells, particularly on MGMT-positive cell populations. VPA itself may induce the apoptosis of glioma cells, and VPA combined with TMZ or ACNU may enhance TMZ/ACNU-induced apoptosis of glioma stem cells. Furthermore, VPA may also promote the methylation of the MGMT promoter to silence MGMT expression in glioma cells, which may be an important mechanism through which VPA enhances the efficacy of TMZ and ACNU in targeting glioma stem cells.

KEYWORDS: O6-methylguanine-DNA methyltransferase; glioma stem cells; valproic acid