Valproic acid induces p21 and topoisomerase-II (alpha/beta) expression and synergistically enhances etoposide cytotoxicity in human glioblastoma cell lines.


OBJECT: Etoposide, a topoisomerase-II inhibitor promotes DNA damage and apoptosis of cancer cells. In this study, we have examined the ability of the histone deacetylase inhibitor, valproic acid (VPA) to modulate gene expression and sensitize glioblastoma cell lines to the cytotoxic effects of etoposide in vitro. METHODS: The effect of VPA and etoposide alone or a combination of the two drugs on the growth of three different glioblastoma cell lines (U87, LN18, and U251) were measured by MTT assays. Drug treated cells were analyzed for their cell cycle profile, gene expression, differentiation status, and induction of apoptosis by flow-cytometry, western blotting, immunofluorescence assays, and caspase activity measurements. RESULTS: We observed that while VPA and etoposide independently inhibited the growth of U87, U251, and LN18 cells, exposure of tumor cells to both drugs significantly enhanced the cytotoxicity of etoposide in all cell lines. VPA promoted a G(1) accumulation of U87, while an increase in the G(2)/M population of U251 and LN18 cells was observed upon exposure to the drug. Treatment with etoposide resulted in a G(2)/M arrest of U87, U251, and LN18 cells, whereas, exposure to both drugs increased the fraction of cells with a G2/M and sub-G1 DNA content. Further, VPA and not etoposide, promoted acetylation of histone H4 and induced the expression of the cyclin-dependent kinase inhibitor (CDKI), p21/WAF1. VPA also up-regulated the expression of the alpha and beta isoforms of topoisomerase-II, as well as the glial differentiation marker, glial fibrillary acidic protein. Finally, a significant increase in caspase-3 activity and apoptosis was observed in the presence of both VPA and etoposide compared to either agent alone. CONCLUSION: Our study demonstrates that VPA sensitizes U87, U251, and LN18 cells to the cytotoxic effects of etoposide in vitro by inducing differentiation and up-regulating the expression of p21/WAF1 and both isoforms of topoisomerase-II.

PMID: 17534580

p21Waf1/Cip1 is a common target induced by short-chain fatty acid HDAC inhibitors (valproic acid, tributyrin and sodium butyrate) in neuroblastoma cells.


Department of Experimental Pathology, University of Bologna, Bologna, Italy.

Histone acetyltransferase and histone deacetylase (HDAC) determine the acetylation status of histones, and thereby control the regulation of gene expression. HDAC inhibitors have been found to inhibit the growth of a variety of tumor cells in vitro and in vivo. We demonstrated previously that the short-chain fatty acid compound butyrate and its derivative tributyrin (both HDAC inhibitors) arrest cell growth and induce differentiation in human neuroblastoma (NB) cells. In the current study we investigated the effect of the HDAC inhibitor valproic acid (VPA) on proliferation and differentiation in human NB cells (SJ-N-KP, AF8). Treatment with VPA resulted in a strong inhibition of cell proliferation and induction of cell differentiation, as revealed by neurite outgrowth and increase of acetylcholinesterase specific activity. Moreover, we addressed the question of whether the cyclin-dependent kinase inhibitors p21(Cip1) and p27(Kip1) are involved in the mechanism of action of members of the short-chain fatty acids class (VPA, sodium butyrate and tributyrin) of HDAC inhibitors, in human NB cells. We demonstrated that p21(Cip1) is a common target of induction of transcription and protein expression for all the three compounds, while only VPA induced a concomitant increase of p27(Kip1) gene expression. These results suggest that p21(Cip1) could be involved in the inhibition of proliferation and induction of differentiation in human NB cells induced by treatment with VPA or tributyrin or sodium butyrate. Moreover, p21(Cip1) could be applied in the molecular monitoring of drug action in the possible therapeutic application of these short-chain fatty acid members of HDAC inhibitors for human NB treatment.

PMID: 15870934