Original Article

Effect of 5-Aza-2'-Deoxycytidine in Comparison to Valproic Acid and Trichostatin A on Histone Deacetylase 1, DNA Methyltransferase 1, and CIP/KIP Family (p21, p27, and p57) Genes Expression, Cell Growth Inhibition, and Apoptosis Induction in Colon Cancer SW480 Cell Line

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Abstract

Background: Cancer initiation and progression depends on genetic and epigenetic alterations such as DNA methylation and histone modifications. Hypermethylation and deacetylation of the CIP/KIP family (p21, p27, and p57) lead to tumorigenesis. Our previous study indicated that DNA methyltransferase (DNMT) inhibitor and histone deacetylase (HDAC) inhibitors can inhibit cell growth and induce apoptosis. The aim of the present study was to investigate the effect of 5-Aza-2'-deoxycytidine (5-Aza-CdR) in comparison to valproic acid (VPA) and trichostatin A (TSA) on HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) genes expression, cell growth inhibition, and apoptosis induction in colon cancer SW480 cell line. **Materials and Methods:** The effect of the compounds on the cell viability was measured by MTT assay. The expression of HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) genes was evaluated by real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). For the detection of cell apoptosis, apoptotic cells were examined by the Annexin V-FITC/PI detection kit. **Results:** The results of MTT assay indicated that 5-Aza-CdR, VPA, and TSA significantly inhibited cell growth ($P < 0.002$, $P < 0.001$, and $P < 0.001$, respectively). The results of real-time RT-PCR demonstrated that all compounds significantly down-regulated DNMT1 and HDAC1, and up-regulated p21, p27, and p57 genes expression. The result of flow cytometry assay revealed that all agents induced apoptosis significantly. **Conclusion:** 5-Aza-CdR, VPA, and TSA can significantly downregulate DNMT1 and HDAC1 and up-regulate p21, p27, and p57 genes expression through which enhance cell apoptosis and cell growth inhibition in colon cancer.

Keywords: *5-Aza-2'-deoxycytidine, cancer, trichostatin A, valproic acid*

Introduction

Chromatin alterations are associated with all stages of tumorigenesis and cancer progression. Cancer initiation and progression depends on genetic and epigenetic alterations such as DNA methylation and histone modifications. Aberrant hypermethylation of the promoter region CpG island in tumor suppressor genes (TSGs) is associated with DNA compaction resulting in transcriptional silencing and cancer induction. DNA methylation is catalyzed by DNA methyltransferases (DNMTs) in mammalian cells. These enzymes are classified into four groups, including DNMT1, DNMT3A, DNMT3B, and DNMT3L. The most abundant DNMT involved in the maintenance of DNA methylation is DNMT1. In mammalian cells, the DNMT1 is the major DNMT responsible for DNA maintenance and methylation pattern. Significant overexpression of DNMT1 has been reported in colorectal cancer.[1] In addition to methylation, histone deacetylation is associated with silenced TSGs and tumorigenesis. In general, hyperacetylation of histones increases transcriptional activity, whereas hypoacetylation decreases transcriptional activity and gene expression. Acetylation of the core histone proteins results from the balance between the opposing activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs). HAT neutralizes the positive charge of lysine by acetylation of histone resulting in a

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decreased interaction between histone and DNA and increased transcriptional activity. Just the opposite, transcriptional activities are blocked when the histones are deacetylated by HDAC. The classical HDAC family includes two different phylogenetic classes, namely Class I (HDAC1, 2, 3 and 8) and Class II (HDAC4, 5, 6, 7, 9, and 10).[2] Overexpression of HDAC1 and DNMT1 has been reported in colon cancer.[3] Cell cycle progression is a regulated process that involves several checkpoints that assess extracellular signals and DNA integrity. Cyclins and cyclin-dependent kinases (CDKs) are positive regulators of cell cycle progression; whereas CDK inhibitors (CDKIs) are important negative regulators, they brake cell cycle progression in response to regulatory signals. The abnormal expression of CDKs or CDKIs leads to cancer. There are two types of CDKIs, including the INK family (p16, p15, p18, and p19) and the CIP/KIP family (p21, p27, and p57).[4] HDAC1 and DNMT1 activity can affect the expression of CDKIs by deacetylation and methylation of these genes resulting in genes silenced and cancer induction. Hypermethylation and deacetylation of the CIP/KIP family (p21, p27, and p57) lead to tumorigenesis.[5,6] DNA hypermethylation can be reversed by DNMT inhibitors such as genistein (GEN), curcumin, epigallocatechin-3-gallate (EGCG), resveratrol, withaferin A, and guggulsterone compounds which have the potential to reverse the epigenetic alterations.[7] Our previous study indicated that DNMT inhibitor GEN can inhibit cell growth and induce apoptosis in hepatocellular carcinoma (HCC).[8-10] HDAC inhibitors (HDACIs) are emerging as an exciting new class of potential anticancer drugs for the treatment of hematological and solid cancers that reactivate certain TSGs. These compounds vary in structure and exert anticancer activity such as cell cycle arrest and apoptosis by acetylation of histone and nonhistone proteins. In general, they are classified into short-chain fatty acids (such as valproic acid [VPA] and phenylbutyrate), hydroxamic acids (such as trichostatin A [TSA]), cyclic peptides (such as depsipeptide), and synthetic benzamides (such as MS-275). HDACI TSA was one of the first natural compounds isolated from the actinomycete streptomyces hygroscopicus that was determined to inhibit HDACs.[11] It has been reported that TSA induces apoptosis in colon cancer HCT116 cells.[12] Previously, we reported that VPA and TSA, as HDACIs, can induce apoptosis in colon cancer and HCC, respectively.[13,14] Recently, HDACIs and DNA demethylating agents have become attractive since they are effective on various cancers such as breast, lung, thoracic, and colon cancer cell lines. The treatment of human cancer cells with HDACIs such as TSA, depsipeptide (FR901228, FK228), sodium butyrate, suberoylanilide hydroxamic acid and VPA and also DNA demethylating agent such as 5-Aza-2'-deoxycytidine (5-Aza-CdR) has been reported.[15] 5-Azacytidine (5-Aza-CR) and 5-Aza-CdR are two well-known DNMT inhibitors and have been approved by the Food and Drug. As we reported previously, genistein (GEN) and trichostatin can induce the significant apoptotic effect.[8] In this study, we decided to investigate the effect of 5-Aza-CdR in comparison to VPA and TSA on HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) gene expression, cell growth inhibition, and apoptosis induction in colon cancer SW480 cell line.

Materials and Methods

Materials

This study was designed to investigate the effect of 5-Aza-CdR in comparison to VPA and TSA on HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) gene expression, cell growth inhibition and apoptosis induction in colon cancer SW480 cell line. In this regard, the human colon carcinoma SW480 cell line was obtained from the National Cell Bank of IranPasteur Institute. 5-Aza-CdR, VPA, TSA, Roswell Park Memorial Institute (RPMI), and 3 (4,5dimethyl2thiazolyl) 2, 5diphenyl-2Htetrazolium bromide (MTT) were supplied by Sigma–Aldrich (Sigma–Aldrich, Louis, MI, USA). The Annexin V and propidium iodide (PI) apoptosis kit were purchased from Life Technologies. Dimethyl sulfoxide (DMSO) was purchased from Merck Co. Darmstadt, Germany. Real-time polymerase chain reaction (PCR) kits (qPCR MasterMix Plus for SYBR Green I dNTP) and total RNA extraction kit (TRIZOL reagent) were obtained from Applied Biosystems Inc. Foster, CA, USA.

Cell culture

Human colon carcinoma SW480 cells were maintained in RPMI supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 1% antibiotics (penicillin/streptomycin), and grown in a humidified incubator at 37°C containing 5% CO_2. The culture medium was replaced every 2 days. 5-Aza-CdR, VPA, and TSA were dissolved at a concentration of 100 mM in DMSO to prepare a stockwork solution, stored at −20°C, and then diluted in the medium before each experiment to obtain all of the other test concentrations. The final DMSO concentration did not exceed 0.1%, and all control groups were administered 0.1% DMSO concentration.

Cell viability assay

The effect of 5-Aza-CdR, VPA, and TSA on the cell viability was measured by MTT assay. First, the SW480 cells were cultured with the culture medium. When the cells got to their logarithmic phase of growth (80% confluent cells), 4×10^5 cells per well were transferred into 96-well plates and allowed to adhere for 24 h. The drug treatment was performed 24 h after cell seeding, the cells were treated with medium containing different doses of 5-Aza-CdR (0.5, 1, 5, 10, and 20 mM), VPA (0.5, 1, 5, 10, and 20 mM), and TSA (0.5, 1, 5, 10, and 20 mM), except control groups for different time periods, in control experiments equal volume of solvent was added. After

exposure to the various concentrations of the compounds for 24 and 48 h, the cells were detached by trypsinization, and the viable cell population was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

Determination of the genes expression by quantitative real-time reverse transcription-polymerase chain reaction

To determine HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) genes expression, the cells were treated with 5-Aza-CdR (2.5 mM), VPA (5 mM), and TSA (1.5 mM), based on IC_{50} values, for 24 and 48 h, IC_{50} values were calculated by GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). After treatment times, total RNA of the treated and control cells was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol and then pretreated with RNase-free DNase (Qiagen) to remove the genomic DNA before to cDNA synthesis. The RNA concentration was determined using a Biophotometer (Eppendorf, Hamburg, Germany). Total RNA (100 ng) was reverse transcribed to cDNA using the RevertAidTM First Strand cDNA Synthesis Kit similar to our previous work.[16] Besides, the sequences of the primers were obtained from our previous work[16] and other articles[17-19] which their sequences are shown in Table 1.

Flow cytometric analysis of apoptosis

For detection of cell apoptosis, apoptotic cells were examined by the Annexin V-FITC/PI detection kit. The SW480 cells were cultured in 24-well plates at a density of 4×10^5 cells/well and incubated overnight before exposure to medium containing 5-Aza-CdR**,** VPA, and TSA. After 24 h, the cells treated with 5-Aza-CdR (2.5 mM), VPA (5 mM), and TSA (1.5 mM), based on IC_{50} values, for 24 and 48 h. After treatment times, all the adherent cells were harvested with trypsin-EDTA, washed with cold PBS, and resuspended in Binding buffer (1×). Annexin-V-(FITC) and PI were used for staining according to the protocol. Finally, the apoptotic cells were counted by FACScanTM flow cytometer (Becton Dickinson, Heidelberg, Germany).

Table 1: Real-time polymerase chain reaction primers used in the present study

Primer name	Primer sequences (5' to 3')	References
DNMT1 forward	GAG GAA GCT GCT AAG GAC TAG TTC	[16]
DNMT1 reverse	ACT CCA CAA TTT GAT CAC TAA ATC	[16]
HDAC1 forward	CCTGGATACGGAGATCCCTA	[17]
HDAC1 reverse	CCGCAAGAACTCTTCCAACT	[17]
P21 forward	AGG CGC CAT GTC AGA ACC GGC TGG	[18]
P21 reverse	GGA AGG TAG AGC TTG GGC AGG C	[18]
P27 forward	ATG TCA AAC GTG CGA GTG TCT AAC	[18]
P27 reverse	TTA CGT TTG ACG TCT TCT GAG GCC A	[18]
P57 Forward	GCGGCGATCAAGAAGCTGTC	[19]
P57 reverse	CCGGTTGCTGCTACATGAAC	[19]
GAPDH forward	TCCCATCACCATCTTCCA	[19]
GAPDH reverse	CATCACGCCACAGTTTCC	[19]

DNMT1: DNA methyltransferase 1, HDAC1: Histone deacetylase 1

Results

In vitro effects of 5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A on SW480 cells growth

The cells were incubated with different concentrations of 5-Aza-CdR (0.5, 1, 5, 10, and 20 mM), VPA (0.5, 1, 5, 10, and 20 μM), and TSA (0.5, 1, 5, 10, and 20 μM), except control groups, which received equal volume of solvent, for different time periods. Inhibitory effects of these agents were evaluated by MTT assay, as mentioned in the materials and methods section. The results indicated that these compounds can inhibit SW480 cells growth significantly versus control groups. As shown in Figure 1, 5-Aza-CdR, VPA, and TSA significantly inhibited cell growth with all concentrations used ($P < 0.002$, $P < 0.001$, and $P < 0.001$, respectively). IC_{50} values of 5-Aza-CdR, VPA, and TSA were 2.5, 5, and 1.5 μM, respectively.

Effects of 5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A on genes expression

To characterize the effect of 5-Aza-CdR (2.5 μM), VPA (5 μM), and TSA (1.5 μM) on HDAC1, DNMT1, and CIP/KIP family (p21, p27, and p57) genes expression, real-time reverse transcription-PCR was carried out. The results indicated that all compounds significantly down-regulated DNMT1 and HDAC1 and up-regulated p21, p27, and p57 genes expression at different periods (24 and 48 h). Maximal and minimal expression was observed with TSA and 5-Aza-CdR treatment, respectively, as indicated in Table 2 and Figures 2 and 3.

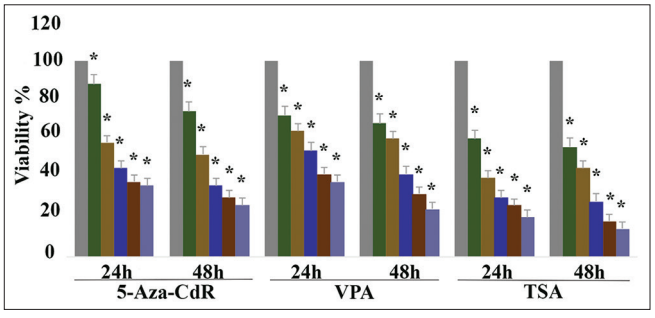

Figure 1: *In vitro* effects of 5-Aza-2'-deoxycytidine (0, 0.5, 1, 5, 10, and 20 μM), valproic acid (0, 0.5, 1, 5, 10, and 20 μM), and trichostatin A (0, 0.5, 1, 5, 10, and 20 μM) on colon cancer SW480 cell viability tested by MTT assay at different times (24 and 48 h). As shown in the figure, the first column of each group belongs to untreated cells (control group), and the others belong to treated cells with the compounds (5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A) at a concentration of 0.5, 1, 5, 10, and 20 μM. Values are means of three experiments in triplicate. Standard errors were <5%. Asterisks (*) indicate significant differences between 5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A treated and the control groups

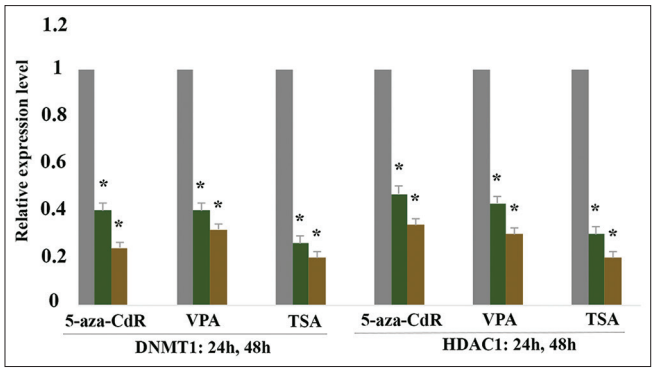

Figure 2: Relative expression level of DNA methyltransferase 1 and histone deacetylase 1 in the experimental groups treated with 5-Aza-2'-deoxycytidine (2.5 μM), valproic acid (5 μM), and trichostatin A (1.5 μM) versus control groups at 24 and 48 h. The first column of each group belongs to untreated cells (control group), and the others belong to treated cells with the compounds with the mentioned concentrations at 24 and 48 h. Asterisks (*) indicate significant differences between 5-Aza-2'-deoxycytidine, valproic acid and trichostatin A treated and the control groups

Effects of 5-Aza-2'-deoxycytidine, valproic acid and trichostatin A on SW480 cells apoptosis

To detect the apoptotic cells by flow cytometry assay, the cells were treated with 5-Aza-CdR (2.5 μM), VPA (5 μM), and TSA (1.5 μM), for different periods, except control groups, and assessed by flow cytometry assay. All compounds induced apoptosis significantly. The percentage of apoptotic cells is indicated in Table 3. Relative analysis between different treated groups at different times indicated that TSA induced apoptosis more significant than two other agents. Maximal and minimal apoptosis was seen in the groups which received TSA (1.5 μM) and 5-Aza-CdR (2.5 μM), respectively, as shown in Figures 4 and 5.

Discussion

Both genetic and epigenetic events control cancer initiation and progression, respectively. Epigenetic changes are heritable and reversible including DNA methylation and histone modifications that are independent of changes in chromatin structure and the primary DNA sequence. The accumulation of epigenetic alterations induces tumorigenesis in normal colonic epithelial cells. Epigenetic drugs, including DNA demethylating agents and HDACIs, can reverse epigenetic events which leads to epigenetic therapy as a treatment option in cancer.[17] Previously, we reported that DNMT inhibitor GEN[20] and HDACI VPA[21] could induce apoptosis in HCC. In this study, we report that TSA, VPA, and 5-Aza-CdR can inhibit cell growth and induce apoptosis in SW480 cells. Similar to our report, it has been reported that TSA induces apoptosis in oral squamous cell carcinoma HSC-3 and Ca9.22 cell lines,[22] prostate cancer (PCa) cell line,[23] human ovarian cancer SKOV3 cells,[24] and human hepatoma cell lines HepG2 and Huh-7.[25] Similarly, it has shown that VPA can induce apoptosis in colon cancer HCT116,[26] breast cancer MCF-7,[27] breast cancer MCF-10A cell line,[28] and ovarian cancer SKOV3 cells.[29] As we reported the apoptotic effect of 5-Aza-CdR, it has demonstrated that 5-Aza-CdR

Table 2: Relative expression level of DNA methyltransferase 1, histone deacetylase 1, P21, P27 and P57 in the experimental groups treated with 5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A versus control groups

Gene	Drug	Dose (μM)	Duration (h)	Expression	*P*
DNMT1	5-Aza-CdR	2.5	24	0.40	<0.014
DNMT1	5-Aza-CdR	2.5	48	0.24	<0.009
DNMT1	VPA	5	24	0.40	<0.044
DNMT1	VPA	5	48	0.32	<0.026
DNMT1	TSA	1.5	24	0.26	<0.046
DNMT1	TSA	1.5	48	0.20	<0.034
HDAC1	5-Aza-CdR	2.5	24	0.47	<0.003
HDAC1	5-Aza-CdR	2.5	48	0.34	<0.001
HDAC1	VPA	5	24	0.43	<0.016
HDAC1	VPA	5	48	0.30	<0.006
HDAC1	TSA	1.5	24	0.20	<0.001
HDAC1	TSA	1.5	48	0.14	<0.001
P21	5-Aza-CdR	2.5	24	1.3	<0.034
P21	5-Aza-CdR	2.5	48	1.4	<0.006
P27	5-Aza-CdR	2.5	24	1.7	<0.002
P27	5-Aza-CdR	2.5	48	1.9	<0.001
P57	5-Aza-CdR	2.5	24	2	<0.002
P57	5-Aza-CdR	2.5	48	2.2	<0.001
P21	VPA	5	24	1.7	<0.044
P21	VPA	5	48	1.9	<0.010
P27	VPA	5	24	2.2	<0.006
P27	VPA	5	48	2.5	<0.002
P57	VPA	5	24	2.6	<0.005
P57	VPA	5	48	2.8	<0.004
P21	TSA	1.5	24	2	<0.046
P21	TSA	1.5	48	2.1	<0.039
P27	TSA	1.5	24	2.3	<0.034
P27	TSA	1.5	48	2.7	<0.010
P57	TSA	1.5	24	2.8	<0.014
P57	TSA	1.5	48	3.1	<0.007

P<0.05 was considered statistically significant. VPA: Valproic acid, TSA: Trichostatin A, HDAC1: Histone deacetylase 1, DNMT1: DNA methyltransferase 1, 5-Aza-CdR: 5-Aza-2'-deoxycytidine

Table 3: The percentage of apoptotic cells treated with 5-Aza-2'-deoxycytidine, valproic acid, and trichostatin A

Drug	Dose (μM)	Duration (h)	Apoptosis (%)	*P*
5-Aza-CdR	2.5	24	13.43	<0.008
5-Aza-CdR	2.5	48	20.29	<0.001
VPA	5	24	22.6	<0.001
VPA	5	48	32.9	<0.001
TSA	1.5	24	43.2	<0.001
TSA	1.5	48	47.8	<0.001

P<0.05 was considered statistically significant. VPA: Valproic acid, TSA: Trichostatin A, 5-Aza-CdR: 5-Aza-2'-deoxycytidine

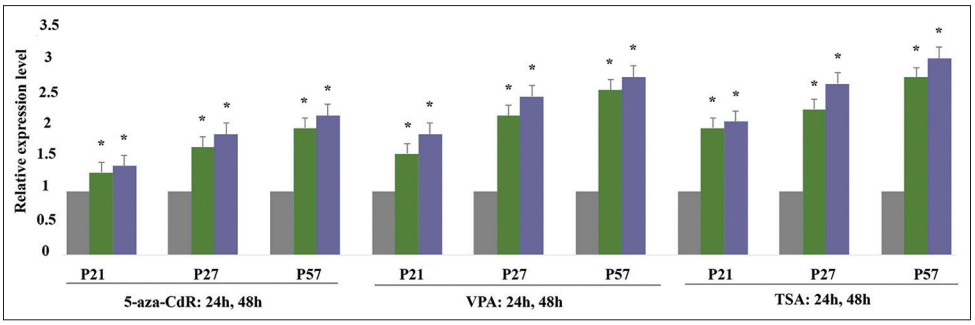

Figure 3: Relative expression level of P21, P27, and P57 in the experimental groups treated with 5-Aza-2'-deoxycytidine (2.5 μM), valproic acid (5 μM), and trichostatin A (1.5 μM) versus control groups at 24 and 48 h. The first column of each group belongs to untreated cells (control group), and the others belong to treated cells with the compounds with the mentioned concentrations at 24 and 48 h. Asterisks (*) indicate significant differences between 5-Aza-2'-deoxycytidine, valproic acid and trichostatin A treated and the control groups

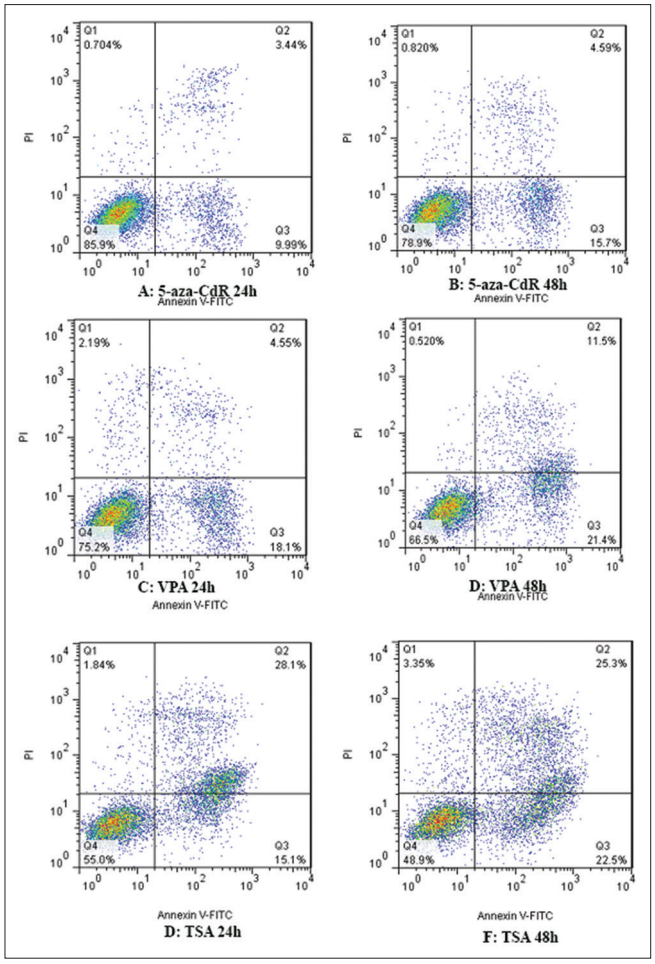

Figure 4: The apoptotic effects of 5-Aza-2'-deoxycytidine (2.5 μM), valproic acid (5 μM), and trichostatin A (1.5 μM) versus control groups at 24 and 48 h on colon cancer SW480 cell apoptosis. The cells were treated with the compounds for 24, and 48 h and the apoptosis-inducing the effect of the agents was investigated by flow cytometric analysis. The upper right quadrant shows the percentage of cells in late apoptosis, the lower right quadrant shows the percentage of cells in early apoptosis, the upper left quadrant shows the percentage of necrotic cells, and the lower left quadrant shows the percentage of viable cells. Results were obtained from three independent experiments and were expressed as mean ± standard error of the mean

can induce apoptosis in human Caco-2 colonic carcinoma cell line,[30] human HCC cell line Huh7,[31] HCC cell line SMMC-7721 and HepG2,[32] and Hela cells.[33]

HDACIs activate several molecular mechanisms by which induce apoptosis. It has been reported that TSA increases JNK phosphorylation thereby down-regulates MCM-2 in colon cancer HCT116 cells.[34] In colorectal cancer SW480, TSA can decrease HDAC1 and HDAC2.[35] In these cancer cells, TSA acts by the mechanisms that regulate suppressors of cytokine signaling (SOCS) and SHP1 genes, negative regulators of JAK/STAT signaling. This drug involves in the regulation of SOCS1 and SOCS3 which significantly downregulate JAK2/STAT3 signaling in this cell line. These findings indicate a mechanistic link between the inhibition of JAK2/STAT3 signaling and the anticancer action of TSA in colorectal cancer.[36] In colon cancer HT-29 cells, HDACI TSA-mediated growth inhibition is associated with downregulation of cyclin B1 mRNA levels. Furthermore, TSA blocks SW1116 and Colo-320 colon cancer cell lines, mainly in the G1 phase. In these cell lines, it increases the p21 (WAF1) gene expression by acetylation of the gene-associated histones and induces cell cycle arrest in the G1 phase.[37]

Several studies have reported that HDACI VPA acts through direct inhibition of HDACs, the enzymes which are responsible for the deacetylation of nucleosomal histones– mainly histones H3 and H4. It can inhibit class I HDACs (HDACs 1–3) and class II HDACs (HDACs 4, 5, and 7).[38] Other mechanisms of VPA include downregulation of protein kinase C activity, inhibition of glycogen synthase kinase-3 β, activation of the peroxisome proliferator-activated receptors gamma, and inhibition of HDACs.[39]

In the current study, we indicated that DNA demethylating agent 5-Aza-CdR inhibited cell growth and induced apoptosis in colon cancer SW480 cell line. Similarly, it has been demonstrated that 5-aza-CR induces apoptosis in colon cancer HCT-116. The mechanism by which this agent induces apoptosis in this cell line includes the up-regulation of genes promoting apoptosis (p53, Bak1, RIPK2, caspase 5, and caspase 6) or cell cycle arrest (GADD45 and $p21^{WAF1}$). This compound reactivates the $p16^{INK4}$ gene expression. Besides, it decreases DNMT1 and DNMT3a

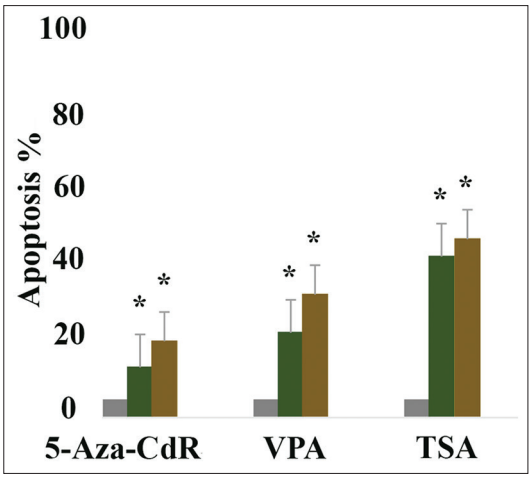

Figure 5: The comparative effects of 5-Aza-2'-deoxycytidine (2.5 μM), valproic acid (5 μM), and trichostatin A (1.5 μM) on colon cancer SW480 cell apoptosis at 24 and 48 h. The first column of each group belongs to untreated cells (control group) and the others belong to treated cells with the compounds with the mentioned concentrations at 24 and 48 h. Asterisks (*) indicate significant differences between 5-Aza-2'-deoxycytidine, valproic acid and trichostatin A treated and the control groups. As shown above, trichostatin A indicated a more significant apoptotic effect in comparison to other agents

mRNA expression. However, the 5-aza-CR action in colon cancer HCT-116 line is mediated by p53 and its downstream effectors GADD45 and $p21^{WAF1}$.[40] It has been demonstrated that 5-aza-dc suppresses cell growth of colorectal cells and induces G2 cell cycle arrest and apoptosis through the regulation of JAK2/STAT3/STAT5 signaling including $p16^{ink4a}$, $p21^{waf1/cip1}$, $p27^{kip1}$, and Bcl-2.[41] In the current study, we report that 5-Aza-CdR**,** VPA, and TSA down-regulated DNMT1 and HDAC1 and up-regulated p21, p27, and p57 gene expression. In line with our report, it has been demonstrated that DNA demethylating agents EGCG and GEN inhibit HDAC1 in the human colon carcinoma cell line HT29.[42] Similarly, it has been reported that GEN and daidzein can reactivate several TSGs by demethylation and acetylation in breast cancer MCF-7 and MDA-MB 231.[43]

Other researchers have shown that 5-Aza-CdR can inactivate all DNMT isoforms, cause demethylation, and reactivation TSGs leads to block cancer growth.[44] HDACI VPA has been reported that modulates histone modification and DNA methylation. It can reprogram certain promoter regions by demethylation. Besides, it can modulate the acetylation level of histone 4 in the promoter region of THBS1 and RASSF1A genes in HCC.[45] In the case of TSA, several studies have shown that TSA can induce DNA demethylation even in the absence of DNA demethylating agents. Besides, it can act synergistically with the DNMT inhibitor 5-Aza-CdR.[46] Similar our result about 5-Aza-CdR as DNMTI, it has been reported that 5-Aza-CdR inactivates the DNMT enzyme, inducing p21Waf1/Cip1 re-expression in the cells that are hypermethylated in the promoter region of the p21 gene.[47,48] TSGs that are shown to increase their expression in response to 5-Aza-CdR exposure include p15, p16, p21, p27, p53, BRCA1, and BRCA2, Apaf-1, and PTEN.[49] Several experimental works have shown that VPA increases the expression of $p21^{CIP1}$, and this effect is associated with apoptosis induction and the cell growth inhibition in HCC HepG2 cells.[50] It has been reported that VPA treatment leads to accumulation of acetylated histones and influences p21 and p27 reactivation in myeloma cell lines OPM-2 and NCI-H929.[51] In PCa cells, VPA and TSA induce apoptosis by upregulating p21/Waf1/CIP1.[52] Several works have demonstrated that HDACs (Class I/II) inhibition by VPA treatment induces a remarkable accumulation of p57Kip2 in colon cancer HT-29 and $CaCO_2$.[53] Besides, it has been shown that TSA induces apoptosis and inhibit cell growth in ACHN renal cell line by p27 up-regulation.[54]

Although TSA, VPA, and 5-Aza-CdR have shown considerable apoptotic and inhibitory effects by down-regulation of HDAC1 and DNMT1 and up-regulation of p21, p27, and p57 gene in this study, it is necessary to evaluate the multiple mechanisms and pathways through which these compounds play their role in colon cancer SW480 cell line.

Conclusion

Our findings indicated that 5-Aza-CdR, VPA, and TSA significantly down-regulated DNMT1 and HDAC1 and up-regulated p21, p27, and p57 gene expression through which enhanced cell apoptosis and cell growth inhibition in colon cancer SW480 cell line suggesting that these compounds may have wide therapeutic applications in colon cancer treatment.

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Conflicts of interest

There are no conflicts of interest.

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