International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.7, No.2, pp 291-297, 2014-2015

Anticancer effect of sodium valpoate

Ali Esmail Al Snafi*[1], Nahi Y. Yaseen[2], Moslim Mohsin Al Shatry[3]

[1]Dept of Pharmacology- Thi qar College of Medicine - Iraq,
[2]Iraqi Center for Cancer and Medical Genetics,
[3]MSc student, Dept of Biology-Thi qar College of Science – Iraq.

Abstract: Cancer is one of the most common diseases worldwide, and ranks the second most common cause of death following cardiovascular diseases. Chemotherapy is able to kill some cancer cells especially the more rapidly replicating tumor cells, but they were nonspecific, characterized by low therapeutic index and associated with a wide range of side effects. Therefore the anticancer field still searching for treatments to avoid these side effects. The in vitro method was used to investigate the effect of pure sodium valproate on four types of tumor cell lines [HeLa (human cervical cancer cell line, Passages 18-25), Rhabdomyosarcoma (RD, at 75 passages), Ahmad-Majeed- Glioblastoma-Multiform-2005 (AMGM-5, human cerebral glioblastoma multiform at passages 75-84), Ahmed-Mohammed-Nahi-2003 (AMN-3, spontaneous mammary adenocarcinoma at 158 passages) and normal cell line Rat Embryo Fibroblast (REF, at 87 passages)] in different concentrations and at different exposure times by MTT assay. The results showed that sodium valproate induced dose- dependent cytotoxic effects against all the tested cell lines. These effect could be attributed to different mechanisms. Accordingly, sodium valproate should be considered as a good alternative therapy.
Keywords: Sodium valproate, Anticancer, Cell Line, Cervical Carcinoma, Rhabdomyosarcoma, Cerebral Glioblastoma, Mammary Adenocarcinoma.

Introduction

Cancer is one of the most common diseases worldwide, and ranks the second most common cause of death following cardiovascular diseases[(1)]. There were an estimated 14.1 million cancer cases around the world in 2012, of these 7.4 million cases were in men and 6.7 million in women. This number is expected to increase to 24 million by 2035[(2)]. This disease killed thousands of people of different age and sex every year in Iraq[(3)]. The mean of the estimated population of registered cases of cancer was 49.65/100000 (1999-2001), while the numbers of incidence were increased to 61.25/100000 in 2009[(4)]. Cancer treatments continue to represent a major challenge to public health and medical research [(5)].

There is emerging evidence that epigenetics plays a key role in the development of the resistance. Epigenetic regulators such as histone acetyltransferases (HATs) and histone deacetylases (HDACs) play an important role in gene expression[(6)]. Many studies have shown that HDAC inhibitors selectively induce cellular differentiation, growth arrest and apoptosis in cancer cells, making these inhibitors a promising new class of anticancer drugs [(7-9)]. Sodium valproate, sodium salt of valproic acid, is a histone deacetylase inhibitor. Recent evidences suggested that sodium valproate participates in multiple processes to treat cancer i.e. blocking tumor cells signaling, down-regulating the proliferation of tumor cell and the production of vascular growth factor, and inducing cell cycle arrest [(10)].

According to accumulated information referring to the interference of sodium valproate in the pathogenicity of cancer and good pharmacokinetic characteristics of sodium valproate, especially its volume of distribution, the current study is designed to investigate the cytotoxic effect of sodium valproate on cancer cell lines.

Material and Methods

The in vitro method was used to investigate the growth inhibitory effect of pure sodium valproate on four types of tumor cell lines [HeLa (human cervical cancer cell line at 18-25 passages), Rhabdomyosarcoma (RD, at 75 passages), Ahmad-Nahi-Glioblastoma (ANG, human cerebral glioblastoma multiform at passages 75-84) , Ahmed-Mohammed-Nahi-2003 (AMN-3, spontaneous mammary adenocarcinoma at passages 158) and normal cell line Rat Embryo Fibroblast (REF, at passages 87)], in different concentrations and at different exposure times by MTT assay. These cell lines were kindly supplied by experimental therapy department, tissue culture unit/ Iraqi centre for cancer and medical genetics research (ICCMGR) maintained in RPMI- 1640 with 10% FCS and MEM with 10% FCS. Cell lines used in this study were subcultured when the cells in the flask formed confluent monolayer, using the previously described protocol [(11-12)]. The cell viability was determined before studying the cytotoxic effect of the drug on each cell line. Seeding of tryptinized and suspended cells for any cell line in a microtiter plate should be in the range of (104–105) cell /well for the growth cytotoxic assay [(13)]**.** Viable cell was accomplished using trypan blue exclusion. Dead cells take up the dye within a second making them easily distinguishable under the microscope from viable cells which remain unstained as mentioned previously [(14)].

Cytotoxicity assay:

Preparation of drugs stock solution:

Pure sodium valproate was obtained from the state company for drug industries & medical appliances (SDI) - Samarra/ Iraq. Stock solutions of this drug were prepared by dissolving 0.01 g of sodium valproate in 1ml triple distal water and filtered by 0.22µm syringe filter.

Preparation of cell lines for cytotoxic assay:

Cell cultures in microtiteration plate (96 wells) were exposed to sodium valproate at six concentrations during the log phase of growth and the effect was determined after the end of exposure time. The following method was used for cytotoxic assay:

a. Seeding:

After cells in the incubated falcon became monolayer, the confluent monolayer was trypsinzed, then 200 µl of cell suspension seeds in microtitration plates were dispensed into each well, except wells at edges of plate to reduce the edge effect, that every well contain about 104 -105 cells/well and then coved by plate lids and sealed with self adhesive film then shacked gently and returned to the incubator.

b. Incubation:

Microtitration plates were then incubated at 37˚c until the cells reached confluence (i.e., vary according to the types of cell line). After cells attachment, the plate was checked for contamination.

c. Exposure:

When the cells are in the exponential phase exactly in population doubling time (PTD), in which the cells were in its full activity (depending on the growth curve of each cell lines), cells were exposed to six concentration of sodium valproate (1000, 500, 250, 125, 62.5 and 31.25 µg/ml) (Four replicates for each tested concentration). 200µl of maintenance medium added to each well of control group (twelve wells were used).

d. Staining:

Cell viability was measured after 24, 48 and 72 hrs of exposure by removing the medium, adding 28 µl of 2 mg/ml solution of MTT and incubating for 1.5 hrs at 37°C. After removing the MTT solution, the crystals remaining in the wells were solubilized by the addition of 130 µl of Dimethyl Sulphoxide (DMSO) followed by 37°C incubation for 15 min with shaking.

The absorbency was determined on a microplate reader at 550 nm (test wavelength). The inhibiting rate of cell growth was calculated as follow [(15)]:

Inhibition rate = $\frac{\text{mean of control} - \text{mean of treatment}}{\text{mean of control}} \times 100$

Statistical Analysis

The optical density and inhibition rate were analyzed by 2-way analysis ANOVA by IBM® SPSS Statistics program version 20 and data are presented as means ±SD. The level of significance between ODs was assayed by student T-test.

Results

Cytotoxic effects of sodium valproate on cell lines:

Effect of sodium valproate on ANG cell line

When the (ANG) cancer cell line treated with six concentration of sodium valproate (31.25 to 1000 µg/ml) the result showed significant cytotoxic effect (table 1) in time and concentration dependant manner in comparison with control and the growth inhibition rate were (11.824, 19.752, 32.518, 41.293, 48.795 and 57.701 %) at 24hrs, (15.620, 25.569, 37.405, 48.265, 59.283 and 66.415 %) at 48hrs and (24.349, 36.085, 47.783, 60.009, 70.063 and 77.917 %) at 72 hrs respectively. Table (1).

Table 1: Effects of sod. valproate on ANG according to the period of exposure

Conc. µg/ml	**Exposure period**					
	24 hrs		**48 hrs**		**72 hrs**	
	Optical density (mean±SD)	**Inhibition rate (mean±SD)**	**Optical density (mean±SD)**	**Inhibition rate (mean±SD)**	**Optical density (mean±SD)**	Inhibition rate (mean±SD)
Control	0.605±0.030		0.606±0.015		0.594 ±0.020	
31.25	0.527±0.087**	11.824±8.320	0.479±0.030**	15.620±4.775	0.452±0.061***	24.349±15.951
62.5	0.479±0.072**	19.752±5.678	0.405±0.069***	25.569±5.354	0.381±0.055****	36.085±16.190
125	0.401±0.042****	32.518±3.497	0.355±0.030****	37.405±5.949	0.312±0.038****	47.783±11.308
250	0.350±0.051****	41.293±4.202	0.293±0.025****	48.265±4.296	0.239±0.031*****	60.009±6.637
500	0.306±0.062****	48.795±7.256	0.231±0.020*****	59.283±3.774	0.180±0.019*****	70.063±2.776
1000	0.253±0.057*****	57.701±6.582	0.190±0.018*****	66.415±3.555	0.134±0.022*****	77.917±2.565

In comparison with control , * (P< 0.05), ** (P< 0.01), *(P< 0.001), ****(P< 0.0001), *****(P< 0.00001).**

Effect of sodium valproate on AMN-3 cell line

The cytotoxic activity of sodium valproate was evaluated against AMN-3 cell line, as shown in Table (2). Statistically, it exerted a concentration and exposure time dependant cytotoxic effects. The cytotoxicity rates were increased from (2.225, 7.275, 12.155, 16.141, 22.052 and 30.319 %) at 24hr exposure period , to (10.248, 16.991, 25.219, 30.937, 34.993, 41.096 %) at 48hr, and further increased to (24.443, 31.770, 37.201, 44.327, 50.396 and 58.583 %) at 72 hr exposure period , when treated with (31.25, 62.5, 125, 250,500 and 1000 µg/ml) respectively.

Table 2: Effects of sod. valproate on AMN-3 according to the period of exposure

Conc. µg/ml	Exposure period					
	24 hrs		48 hrs		72 hrs	
	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)
Control	0.685±0.075		0.610±0.014		0.657±0.066	
31.25	0.634 ±0.085	2.225±3.784	0.634±0.092**	10.248±8.873	0.554±0.058***	24.443±3.944
62.5	0.591±0.068*	7.275±4.577	0.585±0.077**	16.991±8.936	0.500±0.057****	31.770±4.407
125	0.559±0.053**	12.155±3.453	0.528±0.072***	25.219±6.197	0.460±0.042****	37.201±1.603
250	0.534±0.063**	16.141±3.547	0.490±0.090****	30.937±7.162	0.408±0.049****	44.327±3.346
500	0.497±0.064***	22.052±3.597	0.462±0.093****	34.993±8.351	0.363±0.031*****	50.396±1.203
1000	0.445±0.058****	30.319±3.553	0.419±0.085****	41.096±6.633	0.303±0.027*****	58.583±0.093

In comparison with control , * (P< 0.05), ** (P< 0.01), *(P< 0.001), ****(P< 0.0001), *****(P< 0.00001).**

Effect of sodium valproate on HeLa cell line

When the HeLa cell line exposed to 31.25, 62.5, 125, 250, 500 and 1000µg/ml, the growth rates inhibited by 3.012, 9.311, 13.856, 19.417, 24.511 and 31.243 % respectively after 24 hrs of exposure. The inhibition of growth rates were increased to 8.398, 17.642, 26.015, 33.293, 39.539 and 47.871 % when the exposure time increased to 48 hrs. However, after 72 hrs of exposure, the growth rates inhibition reached 20.271, 29.431, 37.400, 45.404, 55.168 and 64.210 % for the same concentrations respectively. As shown in (Table 3).

Table 3: Effects of sod. Valproate on HeLa according to the period of exposure

Conc. µg/ml	Exposure period					
	24 hrs		48 hrs		72 hrs	
	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)
Control	1.053 ± 0.088		1.053± 0.073		1.105±0.074	
31.25	0.940±0.025	3.012±2.331	0.986±0.127*	8.398±4.466	0.846±0.090**	20.271±9.055
62.5	0.878±0.014*	9.311±3.127	0.887±0.131**	17.642±5.926	0.749±0.089**	29.431±8.491
125	0.835±0.025**	13.856±1.738	0.797±0.106***	26.015±2.403	0.665±0.119****	37.400±9.408
250	0.780±0.024**	19.417±3.084	0.721±0.122****	33.293±4.071	0.580±0.123****	45.404±10.262
500	0.731±0.030***	24.511±2.588	0.657±0.147****	39.539±6.471	0.477±0.115*****	55.168±8.797
1000	0.667±0.041****	31.243±2.975	0.565±0.120****	47.871±4.910	0.379±0.093*****	64.210±5.636

In comparison with control , * (P< 0.05), ** (P< 0.01), *(P< 0.001), ****(P< 0.0001), *****(P< 0.00001).**

Effect of sodium valproate on RD cell line

Table (4) showed that sodium valproate exerted dose and time dependant cytotoxic effects on RD cell line. The inhibition rates for the concentrations of 31.25. 62.5, 125, 250, 500 and 1000µg/ml were 0.192, 3.980, 8.076, 15.270, 20.945 and 27.162 % respectively after 24 hr of exposure. When the exposure time increased to 48 hrs, the inhibition rates for these concentrations reached 0.997, 5.763, 12.220, 18.664, 24.857 and 33.050 % respectively. After 72 hrs exposure the inhibition rates were further increased to 10.382, 16.867, 23.702, 32.982, 38.892 and 44.439 % respectively.

Table 4: Effects of sod. Valproate on RD according to the period of exposure

Concentration µg/ml	Exposure period					
	24 hrs		48 hrs		72 hrs	
	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)	Optical density (mean±SD)	Inhibition rate (mean±SD)
Control	0.588±0.014		0.593±0.017		0.588±0.008	
31.25	0.588 ±0.026	0.192±0.333	0.599±0.021	0.997±1.477	0.546±0.049*	10.382±5.727
62.5	0.562±0.044	3.980±5.377	0.568±0.034	5.763±4.565	0.506±0.020**	16.867±4.058
125	0.532±0.047*	8.076±6.810	0.530±0.051**	12.220±7.713	0.464±0.029**	23.702±6.387
250	0.491±0.052**	15.270±6.867	0.491±0.051**	18.664±8.090	0.408±0.049***	32.982±9.396
500	0.458±0.049**	20.945±7.286	0.454±0.058***	24.857±9.947	0.372±0.042****	38.892±9.396
1000	0.422±0.057***	27.162±8.999	0.404±0.058***	33.050±10.045	0.338±0.037****	44.439±7.551

In comparison with control , * (P< 0.05), ** (P< 0.01), *(P< 0.001), ****(P< 0.0001), *****(P< 0.00001).**

Effects of sodium valproate on REF cell line

Table (5), demonstrated the results of cytotoxic effects of six concentrations of sodium valproate after 72 hrs. All concentration of sodium valproate, 31.25. 62.5, 125, 250, 500 and 1000µg/ml, exerted a slight growth inhibition rates (1.335, 5.179, 11.611, 15.125, 22.192 and 26.018 % respectively) after 72 hrs exposure in comparison with control.

Table 5: Effect of sod. Valproate on REF after exposure for 72 hrs

Conc. µg/ml	Exposure period for 72 hrs	
	72 hrs	
	Optical density (mean±SD)	Inhibition rate (mean±SD)
Control	0.859±0.028	
31.25	0.837 ±0.095	1.335±1.269
62.5	0.791±0.086*	5.179±2.092
125	0.738±0.091**	11.611±4.929
250	0.709±0.091**	15.125±5.049
500	0.651±0.099***	22.192±6.897
1000	0.620±0.108***	26.018±8.448

In comparison with control , * (P< 0.05), ** (P< 0.01), *(P< 0.001), ****(P< 0.0001), *****(P< 0.00001).**

Discussion

Malignant cells gain various phenotypic characteristics during the development of cancer, which permit them to proliferate abnormally and eventually invade surrounding tissues. Numerous studies have demonstrated the importance of epigenetic alteration in cancer onset. This has raised the possibility of controlling transcription as a potential approach in cancer therapeutics [(16)].

This study showed that sodium valproate exerted strong significant dose dependent cytotoxic effects on all cell lines. A common mechanism contributing to the antitumor effects of an analog of the natural short chain fatty acids valeric acid (including valproic acid) is their inhibitory effects on several enzymes and signal transduction pathways [(17-20)].

In general this study showed that sodium valproate has direct effect on cell lines this effect could be attributed to its action as histone deacetylases (HDACs), this enzyme regulates the expression and activity of numerous proteins involved in both cancer initiation and cancer progression by removing of acetyl groups from histones. HDACs inhibtor create a non permissive chromatin conformation that prevents the transcription of genes that encode proteins involved in carcinogenesis. In addition to histones, HDACs inhibition, the drug bound and deacetylate a variety of other protein targets including transcription factors and other abundant cellular proteins implicated in control of cell growth, differentiation and apoptosis [(21)].

In previous study, Papi *et al.*, [(22)] evaluated the effect of VPA in inducing growth arrest in G361 melanoma, U87MG glioblastoma and SKNMC A skin tumor cells. In all three cell lines, treatment with VPA caused a pronounced dose dependent growth inhibition. This effect was accompanied by an apoptotic action. During apoptosis, the effects of phosphatidylserine (PS) residues at the outer plasma membrane have been demonstrated to occur early in the apoptotic process preceding the loss of plasma membrane integrity and DNA fragmentation [(23)]. In particular, they found that exposure to VPA enhanced the expression level of *Bax gene*, whereas it induced down regulation of Bcl2. Their observations regarding VPA induction of pro apoptotic activity and modulation of apoptosis related proteins were confirmed the previously demonstrated role of VPA and other HDAC inhibitors in apoptotic pathways in cancer cells [(24-25)].

Otherwise, VPA had been shown to induce the depletion of proteins that maintain chromatin structure in breast MCF-7 cancer cells thus leading to the potentiation of DNA damaging agents of other drugs [(26)]. Olsen *et al.*, [(27)] find that estrogen receptor of estrogen stimulated human breast cancer cells (MCF-7) were inactivated by the therapeutic concentrations of VPA, it also inhibited cell growth by mechanisms do not involve the estrogen receptor. Other study showed that VPA exerted reversible cell cycle arrest by modulation of heterochromatin maintenance proteins, which represented an important additional step in the control of the chromatin structure and its access to regulatory factors. VPA induced chromatin decondensation led to enhanced sensitivity of DNA to nucleases and increased DNA binding by intercalating agents [(26)]. Accordingly, it appeared that VPA exerted cytotoxic effects by different mechanisms, it also potentiate the effect of other cytotoxic drugs.

Conclusion:

According to our results , we can conclude that the good pharmacokinetic characteristics, safety and direct broad anticancer effects make sodium valproate a valuable additional anticancer treatment.

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