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Evaluation of Valproic acid effects on K562 cells: In vitro Study

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ABSTRACT

Introduction: Valproic acid operates as an inhibitor of Histone deacetylase (HDAC). HDACs have high remark on cancer cells and conduce to change of remarks and activities of those proteins involved in carcinogenesis and inspire apoptosis which is in malignant cells in vitro. Alsovalproicacid induces differentiation and apoptosis in malignant cells, since it may be used to decrease tumor cell growth. Theaim of this study was to investigate the effects of valproic acid on cell growth and apoptosis of K562 cells(the member of cancer cells with myeloid origin) in vitro.
Methods: For this purpose, K562 cells were cultured and then different concentrations of the drug of valproic acid prepared and its antitumor properties at 24, 48 and 72 hours after treatment were measured by MTT assay. In the next stage, DNA electrophoresis and staining with Hoechst was used to analysis of apoptotic cells in vitro.
Results: The results showed that the antitumor effects of valproic acid increased in a dose- and time- dependent manner. As well as, the apoptotic effects of this drug were indicated by the results of electrophoresis. On the other hand, the maximum inhibitory effect was observed in higher concentration and 72 hours after treatment. The IC50 of valproic acid was also calculated 80 µM by Compusyn software.
Conclusion: based on obtained results we conclude that the valproic acid has suitable application for inhibiting of K562 cells, and these antitumoric properties would warrant further studies on the clinical application of valproic acid. Therefore, this drug might be effective for the prevention and treatment of chronic myeloid leukemia.
KEY WORDS: K562 cell line, Valproic acid, Antitumoric properties, Apoptosis, MTT assay, In vitro.

1.INTRODUCTION

Valproic acid inhibits the histonede acetylases and induces differentiation and apoptosis in malignant cells, since it may be used to treat cancer. Its primary use in medicine is in the treatment of epilepsy, bipolar mania and migraine prophylaxis [1]. Recently, it has been suggested for treatment of HIV and cancer, owing to its histone deacetylase-inhibiting effects[2]. Although the mechanism of action of valproate is not fully understood, [3] it has been recently shown to protect against a seizure-induced reduction in phosphatidylinositol (3,4,5)-trisphosphate (PIP3) as a potential therapeutic mechanism[4]. It also possesses histone deacetylase-inhibiting effects. The inhibition of histone deacetylase, by promoting more transcriptionally active chromatin structures, likely presents the epigenetic mechanism for regulation of many of the neuroprotective effects attributed to valproic acid. Intermediate molecules mediating these effects include VEGF, BDNF, and GDNF [3]. It seems, the most effective anti-epileptic, is its 5 – 8 atomic chain length. Amides and valproic acid esters are antiepileptic active drugs [3] and valproic acid operates as an inhibitor of HDAC. HDACs have high remark on cancer cells and conduce to change of remarks and activities of those proteins involved in carcinogenesis and inspire apoptosis which is in malignant cells in vitro. Several observations shows that unusual activity of HDACs causes to repress of duplication of tumor repressor genes that this process has an essential role in improving of tumor. So many observations suggest that general acetylation of histones may be involved in aggression and metastasis of cancer cells[5]. Locemy or leukemia is a kind of common and deadly cancers[6]. Locemy, the cancer of blood maker contextures, includes marrow and lymphatic system and it is made by the white blood cells and lymph. White blood cells usually grow and disport controlled and methodical when the body needs them. But in locemy, this process is disordered and the growth of the blood cells is uncontrollable [7]. Persistent myeloid locemy is one of the most known kinds of locemy which engenders in multipower fundamental cells because of two sided relocation between ab1 gene in chromosome 9 and bcr in chromosome 22. The result of this choromosomic relocation, forms the Ab1- Bcr fused gene[8]. Also this relocation is known by Philadelphia chromosome which is positive about 95% of patients. The result of this fused gene in CML is p210Bcr –Ab1 protein which is a tyrosine kinase with continuing activity. This protein causes the irregular

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duplication of myeloid cell category and the disorder in death of planning (apoptosis)[9,10]. The K562 cancer cell line is the member of cancer cells with myeloid origin which is derived from a 53-age lady who was stricken ofpersistent leukemia. Cell lines usually are from lymphoblasts which have gotten by companionship with apshtainbar virus and lumphatic markers differentiated[11]. K562 cells are about 1.5 times of ordinary cells with chromosome[12]. K562 cell line has not lymphatic and surface globin – safe features[13].

Apoptosis is programmed cell death that has important roles in physiologic and pathologic conditions. Most of the molecules and signaling pathways which are involved in this process are well known. Cell – death has an important role in natural physiologic control of body and in many pathologic conditions[14].

One of the features of cancer cells is their ability to escape from apoptosis that the result of this process is the parochial of cell – death signalic route. Apoptosis has an important role in cancer therapy since it is a general goal of most therapeutic strategies[15]. Anticancer drugs and chemotherapy facilitates apoptosis in neoblastic cells[16]. The aim of this study was to investigate the effects of valproic acid on cell growth and apoptosis of K562 cells in vitro.

2. MATERIAL AND METHODS

2.1. K562 cancer cell line culture:

K562 cell line was obtained from Pastor Institute of Iran, then cultured in RPMI-1640supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco-BRL) at 37 °C in a humidified incubator with an atmosphere of %95air and 5% CO_2 [17].

2.2. Valproic acid preparation:

The drug was procured from sigma and then their different concentrations(25, 50, 75 and 100 µM) were prepared by dissolving them in DMSO.

2.3. MTT assay:

The K562 cells with 10000 per well were cultured in 96 well plates, and then treated with different concentrations of the valproic acid. After treatment and incubation at 37ºC for 24, 48 and 72 h, the MTT dye was added to the well of 96 well plates and the plates were incubated at 37 ºC for 3h. After incubation, the plates were read by Eliza readers atthe wavelength of 490 nm [17].

2.4. DNA extraction and electrophoresis:

The K562 cells were cultured and treated with the drugs and then centrifuged at 1500 rpm. After centrifugation, the lysis buffer was added to the cell pellets and DNA was extracted by phenol / chloroform / isoamylalcohol.The solutions containing the extracted DNA were analyzed by electrophoresis on the 1%agarose gel at 80 mv for 1 hour with fluorescent staining material of Ethidium bromide. Finally, gels were visualized under UV illumination in a gel documentation system and photographed [18,19,20].

2.5. Hoechst staining:

To each of sinks, 1 ml FBS (Fetal bovine serum)10% medium from 24-room plate was poured and then 1 ml of different doses of valproic acid was added to the sinks. The IC50 of drug was calculated by Compusyn software. The jorums of each sinks after incubation at 37 ºC for 48 h were transferred to microtubesand centrifuged at 2000 rpmfor 5 min. The supernatant was discarded and then 100-200 µlof methanol was added to each alluvium in order to fix the cells and put at 20ºC. After 15 min, the solutions were centrifuged at 1000 rpm for 10 min. The supernatants were discarded and then 50µl of PBS buffer and 2 µl of fined Hoechst color (1 µl Hoechst and 100 µl PBS buffer) were added to each alluvium. Samples were incubated at the room temperature for 10 min and then centrifuged at 1000 rpm for 10 min.After discarding of supernatants,100 µl of PBS buffer was added to each alluvium and completely stirred until achievement to an invariable sample. We put one drop of gotten samples on the microscope slides and the glass coverslips were put on them. The prepared microscope slides were transported to the fluorescence microscope for evaluation and photography (21).

2.6Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard error (SE) of the mean or standard deviation (SD) of the mean significant differences between means were separated by analysis of variance followed by Tukey's test at the 5% level.

3. RESULTS

3.1. Cell-mortifying activity of different concentrations of valproic acid

The results of this study showed that the samples treated with valproic acid have cell-mortifying activity compared to control group without any valproic acid treatment. Also based on these results, the level of cell-

mortifying decreased when the density of valproic decreased. Also, we observed that the cell-mortifying activity of valproic acid was dose-and time-dependently. The maximum effect of valproic acid was observed in 100 µl after 72 h treatment by using the MTT assay (Fig1).As well as, the maximum cytotoxicity of valproic acid was %68.3. Also, the IC50 of valproic acid was 80 µM.

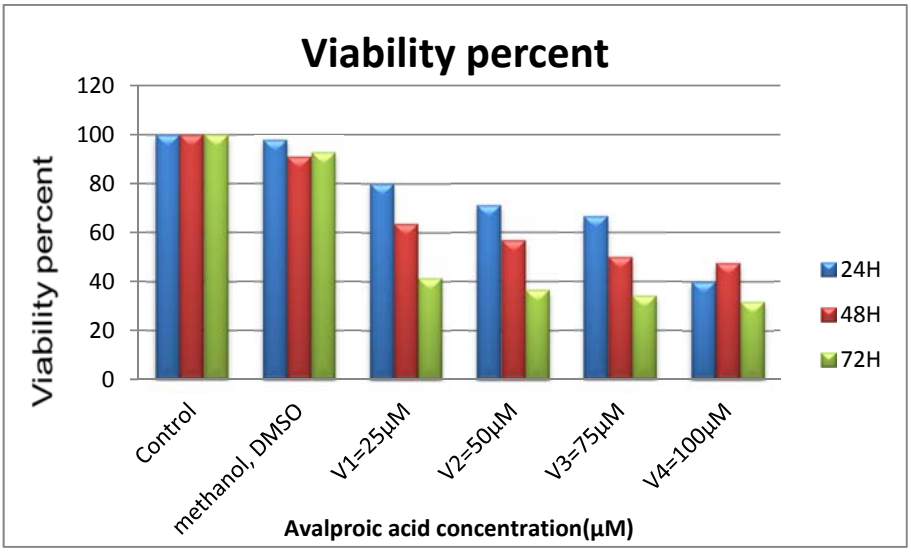


Fig.1. Effects of different concentrations of valproic acid on cell-mortifying activity of K562 cells.

3.2.Evaluation of K562 apoptosis level

3.2.1.Apoptosis surveying of valproic acid by DNA gel electrophoresis

The results showed that the mentioned drugs bind to K562 cells. So they cause cell-mortifying by inspiring apoptosis. The creation of band and the fragmentation of DNA are demonstrators of apoptosis that this condition is created after using of the valproic acid (Fig 2-2).

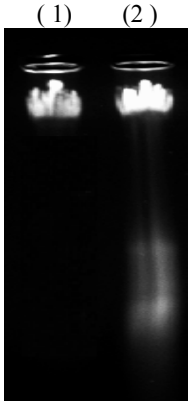


Fig.2. Survey of cell-dying condition, K562 cells which are under attendance by valproic acid. Lane 1: Control. Lane 2: IC_{50}valproic acid

3.2.2.Surveing of morphological revolution of K562 cells by Hoechst staining

The figure3 indicates the photograph of treated K562 cells with IC50 concentration of valproic acid and 100 µmol of synthetic drug under fluorescence microscope after Hoechst staining. Morphological changes observed in treated cells confirmed the cellular apoptosis. In this staining, natural cells (Fig. 3A) are being seen by invariable blue while the core of cells witch are exposed by apoptosis, are visible disorderly like shiny spots by agglomerating the chromatin and fragmentation of core (Fig. 3B).

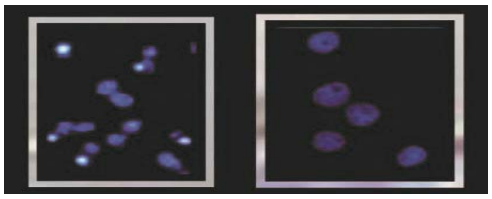
B  A

Fig.3. TheK562 cells staining by Hoechst color under fluorescence microscope. A: non-treated cells. B: treated cells with valproic acid.

4. DISCUSSION

In this study, we evaluated the effects of valproic acid (VPA) on cell growth and apoptosis of cancerous K562 cell line in vitro. Valproic acid is an effective inhibitor of HDACs that directly inhibits the HDAC enzymatic activity at 0.5 mM concentration(22).In this study, according to the results of MTT assay, DNA electrophoresis and Hoechst staining, the VPA treatment abolished the K562 cells' growth and induced the apoptosis. Previously, Phiel et al. indicated the ability of VPA in activation of transcription regulated multiple promoters and demonstrated the over-expression of HDAC1 in HeLa cells at presence of VPA. It may be possible that the antitumoric effects of VPA are related to its inhibitory effects on HDAC1 expression in K562 cells at present study.

Also, we observed the time- and dose-dependent effects of VPA on cell-mortifying in vitro. The IC50 dose of VPA used in this study was in therapeutic range of VPA therapy in humans. According to previous studies(22), 1mM valproic acid induces both apoptosis and cell cycle arresting in thyroid cancerous cells. Also, it has been shown that VPA counters whit differentiation of tumor cells in thyroid carcinoma models. The treatment with VPA at doses between 0.5 and 2 mM in one papillar and in three follicular thyroid cancer cell lines lead to reductionof26–59% of cells' growth, and up to 77% at 72 h.Also, the studies about the effects of VPA on prostate androgen receptors demonstrated that the treatment of1.2–5 mMVPA for 3 days lead to reduce the survival of cells through the decreasing of proliferation and the increasing of apoptosis. Until now, the effects of valproic acid have been studied in neuroblastoma, breast, glioma, colon, hepatocarcinoma, cervix, endometrial, melanoma, ovarian, neuroec to dermal, teratocarcinoma, fibrosarcoma and several other cancer (22); however, this was the first time that the antitumoric effects of VPA was evaluated on cancerous K562 cell line, the member of cancer cells with myeloid origin.

In the present study, it has been indicated that valproic acid significantly ($p<0.05$) inhibited the cell growth in all concentrations and in all time intervals. The results showed that the antitumor effects of valproic acid increased in a dose- and time- dependent manner with a maximum effect in 100 μM after 72 h.

5. Conclusion

In this study, the results showed that valproic acid have anti-cancer property. Also we showed drug causes apoptosis of cancer cells, although to determine the exact percent of apoptosis the quantitive methods like flowcytometry is essential. As well as, our experiments demonstrated that the effects of VPA follow a time- and dose-dependent manner.

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