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Research Article

Sodium Valproate Can Result in Displaying Cell Nucleus Outline of Living A549 Cells and Hela Cells Under White Light

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Abstract

Treating A549 or Hela cells with sodium valproate at concentrations of 1.8 or 3 mmol/L for 72 hours resulted in significant nuclear like outline under white light and inhibition of cell proliferation without staining or labeling. It is also found in this study that the nuclear like outline disappeared and the rate of cell proliferation increased after removing the drug from the culture medium for 48 hours. When A549 cells or Hela cells were cultured continuously for 15 or 20 days without refreshing the medium, these cells showed significant nucleus outline. A549 cells were observed for nuclear shape after acridine orange staining and it confirmed that the nuclear like outline displayed by sodium valproate treatment was indeed the outline of the nucleus. The process of displaying the nucleus outline should be the result of cytoskeleton movement. Further experiments in this study will contribute to understanding the mechanisms of cytoskeleton and be helpful to research about aging or malnutrition.

Keywords: Sodium valproate, Nucleus outline, White light, Cytoskeleton, Aging, Malnutrition

Introduction

Sodium valproate is a nitrogen free broad-spectrum antiepileptic drug that has varying degrees of antagonistic effects on seizures caused by various methods. Subsequently, evidence has been proposed for a wide spectrum of actions of this drug, including antitumoral and neuroprotective properties [1]. Valproate can induce pronounced ultrastructural changes in the population of glial cells and nerve cells of the dentate nucleus of the cerebellum in rats [2]. Effect of of histone deacetylase inhibitors include sodium valproate on the cell nucleus and nucleolus of leukemic myeloblasts in vitro werereasearched. Using simple cytochemical procedures can demonstrate RNA and proteins of silver-stained nucleolar organizers. Silver-stained proteins of nucleolus organiser regions (AgNORs) were visualized by silver reaction under conditions which facilitated to see their distribution by light microscopy [3]. Valproic acid can inhibit the proliferation of A549 cells and effectively inhibit metastasis and treat lung cancer [4]. Extracellular signals can cause rearrangement of the cytoskeleton. It is found that differentiation via Sim Vastatin (SV)-dependent actin cytoskeleton changes was regulated by the extracellular signal-regulated kinase (ERK)-1/2 and p38 kinase pathways [5]. Short-term actin cytoskeleton response to auxin requires AUX1 and/or cytoplasmic auxin [6]. The peripheral nuclear lamina is located near the nuclear inner mem-

brane and consists of lamin filaments and integral membrane proteins. The internal lamins, together with Tpr-based filaments that connect to nuclear pore complexes, are proposed to be major structural elements of the internal nuclear matrix [7]. The nuclear matrix organizes nuclear DNA into operational domains in which DNA is undergoing replication, transcription or is inactive. The proteins of the nuclear matrix are among the most thermal labile proteins in the cell [8]. Cells that proliferated to VHD (very high density, 86,500 cells/cm2) exhibited significantly rounder nuclei than nuclei from all other cell density and seeding protocol groups. In contrast, nuclei from cells that were seeded at the VHD were flatter than nuclei from cells of all other groups. Furthermore, the significant rounding of nuclei in the cells that proliferated to VHD was accompanied by a two-, six-, and ninefold increase from baseline in Runx2, Sox9, and Aggrecan (AGC) expression, markers indicative of precondensation, peri-, and post-condensation events, respectively [9]. Sodium valproate sensitizes non-small lung cancer A549 cells to gammadelta T-cell-mediated killing through upregulating the expression of Major Histocompatibility Complex (MHC) class I chain-related protein A (MICA) [10]. Sodium valproate (VPA) was shown to suppress p16 INK4a, a biomarker gene of cervical carcinoma HeLa cells, and to increase the abundance of the tumor suppressor protein p21WAF1/Cip1, thus contributing to the basic knowledge regarding the antitumorigenic potential of VPA [11]. Valproate can increase triglyceride levels and enhance lipid accumulation in HepG2 cells [12]. Downregulation of matrix metalloproteinases contributes to the inhibition of cell migration and invasion in HepG2 cells by sodium valproate [13].

Materials and Methods

Experimental Materials

1640 medium, DMEM medium, and fetal bovine serum were all purchased from Gibco Co. (Canada). A549 and Hela cells were purchased from Wuhan University China Typical Culture Collection Center. Sodium valproate tablets, 0.2g per tablet, Purchased from Hunan Xiangzhong Pharmaceutical Co., Ltd, batch number: 1H230904.

Cell Culture

Add 10% (V/V) fetal bovine serum and 80 U/mL penicillin streptomycin to the culture medium. The culture medium for A549 cells is 1640; The culture medium for Hela cells is DMEM. Inoculate cells onto a 24 well plate, with cell concentration of 80000/mL approximately.

Cell Experiments

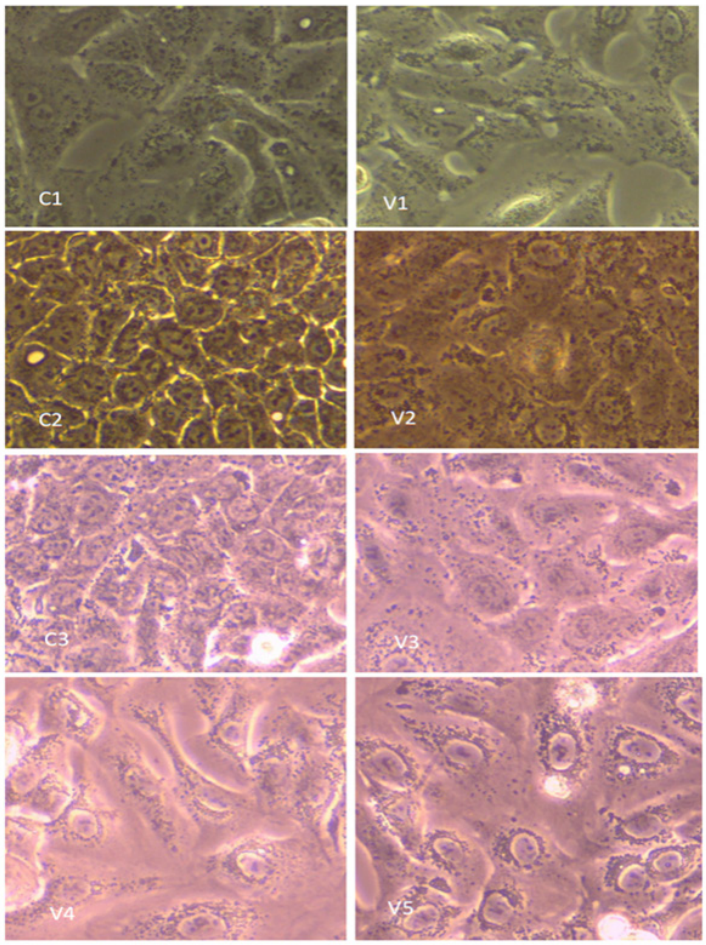
The control group was treated with physiological saline, while the experimental group was treated with Sodium valproate on A549 or Hela cells, with final concentrations of 0.6, 1.8, or 3 mmol/L respectively.

Acridine Orange Staining

Cultivate A549 cells in a 24 well culture plate. Take 10μl of a 100mg/L solution of Acridine Orange (AO) dissolved in PBS and add it to 1ml of culture medium. After 1 minute of treatment, they were washed twice with PBS and then add 0.5ml PBS. Observe and take photos under a fluorescence microscope [14].

Results

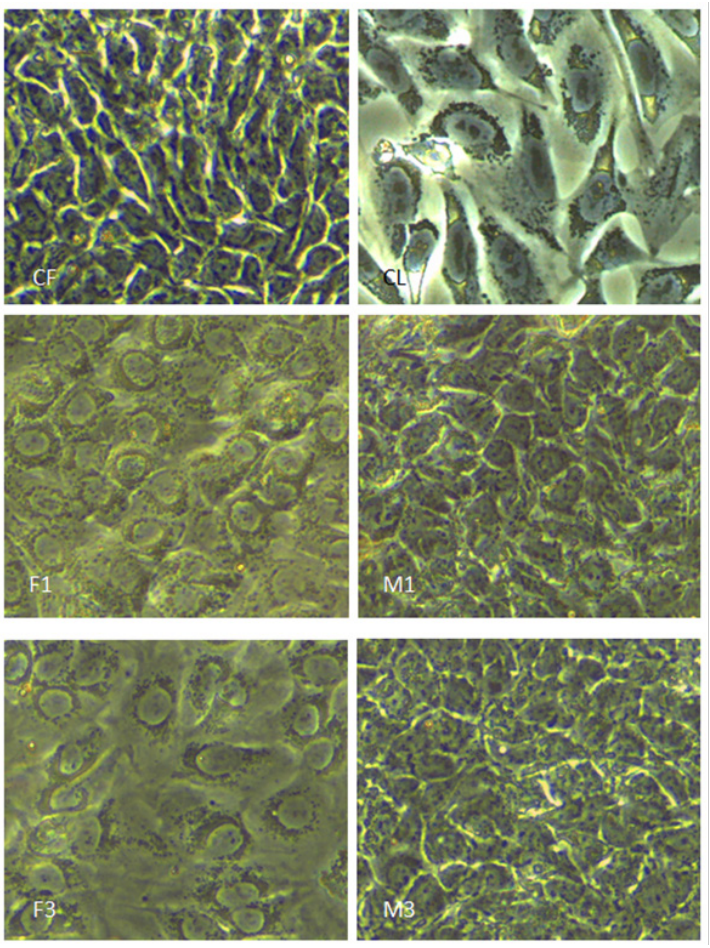
Effects of gradient concentrations of Sodium valproate on A549 cells after 1d, 2d or 3d of treatment After treating A549 cells by sodium valproate with final concentrations of 3 mmol/L for 24 hours, few A549 cells showed significant nucleus outline. After treating A549 cells by sodium valproate with final concentrations of 3 mmol/L for 48 hours, a few A549 cells showed significant nucleus outline. After treating A549 cells by sodium valproate with final concentrations of 0.6, 1.8, or 3 mmol/L for 72 hours, few, a few or almost all A549 cells showed significant nucleus outline respectively. At the same time few or none A549 cells in control team showed significant nucleus outline (Figure 1).



Note*: The morphology of A549 cell treated with sodium valproate (×400).
C1, C2 or C3: control treated with physiological saline; V1: After treatment with sodium valproate at 3mmol/L for 24h; V2: After treatment with sodium valproate at 3mmol/L for 48h; V3: After treatment with sodium valproate at 0.6mmol/L for 72h; V4: After treatment with sodium valproate at 1.8mmol/L for 72h; V5: After treatment with sodium valproate at 3mmol/L for 72h.
Figure 1: Effects of gradient concentration sodium valproate treatment on A549 cells for 24, 48 or 72 hours.

Effects Of Sodium Valproate on A549 Cells After 72 Hours of Treatment and then Treated with Culture Refresh to Remove the Drug for 48 Hours

After the control group and the experimental groups were cultured for 72h, they all refreshed with 1640 culture medium. Then, the control group was added with physiological saline. The experimental groups classified as refreshed drug team and removed drug team. The refreshed drug teams were added with sodium valproate to 1.8 or 3 mmol/L again. The removed drug teams were added with physiological saline instead of sodium valproate. Then, they were observed after cultured for 48h. None of A549 cells in control team showed significant nucleus outline. Almost all of A549 cells in refreshed drug teams still showed significant nucleus outline. While, the A549 cells in removed drug teams no longer showed significant nucleus outline. At the same time, the A549 cells in removed drug teams have much higher proliferation than in refreshed drug teams (Figure 2). In addition, CL is control group cultured continuously for 15 days without refreshing the medium. These A549 cells are aged and malnourished and part of them showed significant nucleus outline without sodium valproate treating (Figure 2CL).

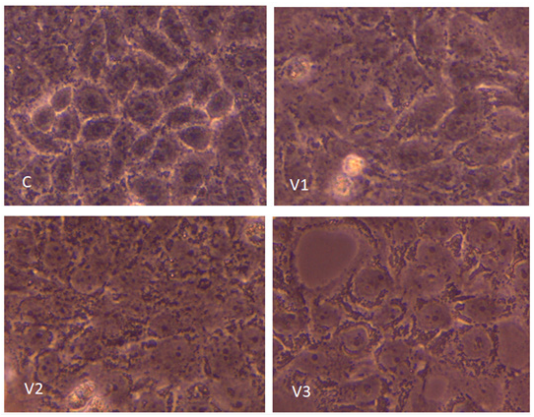


Note*: The morphology of A549 cell treated with sodium valproate (×400) .
CF: Control group with culture medium refreshed
CL: Control group cultured continuously for 15 days without refreshing the medium
F1: were added with sodium valproate to 1.8 mmol/L again.
M1: were added with physiological saline instead of sodium valproate.
F3: were added with sodium valproate to 3mmol/L again.
M3: were added with physiological saline instead of sodium valproate.

Figure 2: Effects of sodium valproate on A549 cells after 72 hours of treatment and then treated with culture refreshed to remove the drug for 48 hours.

Effects of Gradient Concentrations of Sodium Valproate on Hela Cells After 96 Hours of Treatment

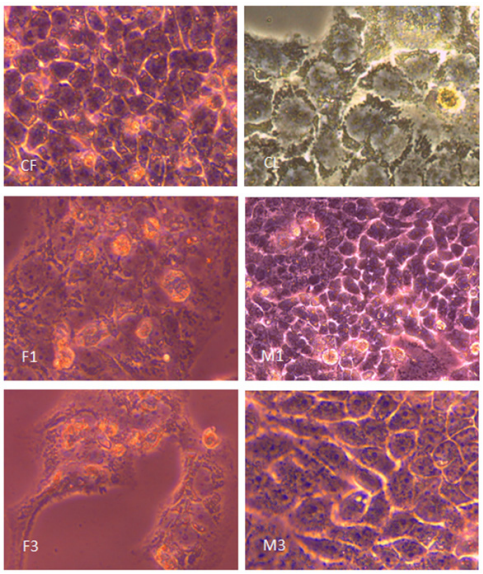
After treating Hela cells by sodium valproate with final concentrations of 0.6 mmol/L for 96 hours, none of Hela cells showed significant nucleus outline. It is similar to control group. After treating Hela cells by sodium valproate with final concentrations of 1.8 mmol/L for 96 hours, a few Hela cells showed significant nucleus outline. After treating Hela cells by sodium valproate with final concentrations of 3 mmol/L for 96 hours, almost all of Hela cells showed significant nucleus outline. At the same time few or none Hela cells in control team showed significant nucleus outline (Figure 3).



Note*: C: control treated with physiological saline; V1: After treatment with sodium valproate at 0.6mmol/L; V2: After treatment with sodium valproate at 1.8mmol/L; V3: After treatment with sodium valproate at 3mmol/L.
Figure 3: Effects of gradient concentration sodium valproate treatment on Hela cells for 96 hours.

Effects of Sodium Valproate on Hela Cells After 96 Hours of Treatment and then Treated with Culture Refresh to Remove the Drug for 48 Hours

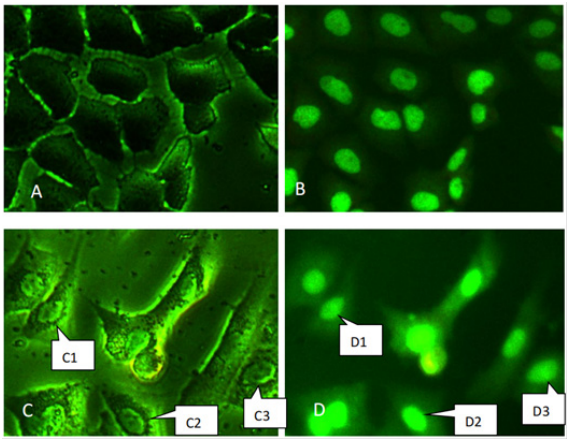
After the control group and the experimental groups were cultured for 96h, they all refreshed with DMEM culture medium. Then, the control group was added with physiological saline. The experimental groups classified as refreshed drug team and removed drug team. The refreshed drug teams were added with sodium valproate to 1.8 or 3 mmol/L again. The removed drug teams were added with physiological saline instead of sodium valproate. Then, they are observed after cultured for 48h. None of Hela cells in control team showed significant nucleus outline. Almost all of the Hela cells in refreshed drug teams still showed significant nucleus outline. While, the Hela cells in removed drug teams no longer showed significant nucleus outline. At the same time, the Hela cells in removed drug teams have much higher proliferation than in refreshed drug teams (Figure 4). In addition, CL is control group cultured continuously for 20 days without refreshing the medium. These Hela cells are aged and malnourished and most of them showed significant nucleus outline without sodium valproate treating (Figure 4CL).



Note*: The morphology of Hela cell treated with sodium valproate (×400) .
CF: Control group with culture medium refreshed
CL: Control group cultured continuously for 20 days without refreshing the medium
F1: were added with sodium valproate to 1.8 mmol/L again.
M1: were added with physiological saline instead of sodium valproate.
F3: were added with sodium valproate to 3mmol/L again.
M3: were added with physiological saline instead of sodium valproate.
Figure 4: Effects of sodium valproate on Hela cells after 96 hours of treatment and then treated with culture refreshed to remove the drug for 48 hours.

Comparion of Cell Nucleus Displayed by Sodium Valproate or Acridine Orange Staining

C1 cell is D1 cell, C2 cell is D2 cell and C3 cell is D3 cell. C1, C2 and C3 cells were observed under normal light. While, D1, D2 and D3 cells were observed under fluorescence. After comparison, it can be seen that the shape of the cell nucleus displayed by C1 sodium valproate is the same as that displayed by D1 acridine orange staining. C2 sodium valproate staining showed that the shape of the cell nucleus was the same as D2 acridine orange staining. C3 sodium valproate staining showed that the shape of the cell nucleus was the same as that of D3 acridine orange staining. It can be seen that the suspected structure of the nucleus displayed after treatment with sodium valproate is indeed the nucleus (Figure 5).



Note*: The morphology of A549 cell treated with sodium valproate and acridine orange (×400).
A: The image of A549 cells in the control group stained with acridine orange under normal light;
B: The image of the same part of A under a fluorescence microscope;
C: The image of A549 cells in the experimental group treated with 3mmol/L sodium valproate for 72 hours, stained with acridine orange, under normal light;
D: The image of the same part of C under a fluorescence.
Figure 5: Comparison of cell nucleus displayed by sodium valproate and acridine orange staining for cell nucleus.

Discussion

As results shown, after treating A549 cells or A549 cells by sodium valproate with final concentrations of 3 mmol/L for 72 or 96 hours, almost all cells showed significant nucleus outline under white light without staining or labeling. At the same time few or none A549 cells or Hela cells in control team showed significant nucleus outline. They are observed by white light microscope. Usually, nucleus outline were observed by staining. Up to now, the paper about nucleus outline in living A549 cells or Hela cells being observed by white light without staining was not found. Acridine orange staining confirmed that the suspected nuclear structure displayed after treatment with sodium valproate was indeed a nucleus. The process of displaying cell nucleus outline takes more than 72 hours, which may be the result of the movement of the cytoskeleton and nuclear cytoskeleton. After the appearance of structures resembling nuclear outlines, the culture medium was refreshed to remove the drug, and then cultured for 48 hours. The structures resembling nuclear outlines displayed had disappeared and cell proliferation activity was restored. It can be seen that the phenomenon of cell outline appearing can be reversed, that is, the cytoskeleton and nuclear cytoskeleton undergo opposite changes.

In addition, when A549 cells or Hela cells were cultured continuously for 15 or 20 days without refreshing the medium, these cells are aged and malnourished and part of them showed significant nucleus outline without sodium valproate treating (Figure 2CL). So, this finding may be helpful to research about aging or malnutrition. Further ultrastructural and molecular mechanism studies by on the nucleus outline or its disappearance induced by sodium valproate are beneficial for a deeper understanding of movement of cytoskeleton, as well as for a deeper understanding of cellular biological activities [15,16].

Article Information

Competing Interests

We authors declare no competing interests.

Author Contributions

Jinhui Shao designed the study. Jinhui Shao collected and analyzed the data. Jinhui Shao wrote and revised the manuscript. The author has read and approved the submission of the manuscript.

Acknowledgments

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Data availability

Data will be made available on request.

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