

Effects of Sodium Valproate on the Replication of Herpes Simplex Virus Type 1: An in Vitro Study

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Abstract

Background: Sodium valproate, an anticonvulsant drug, is reported to stimulate Human Immunodeficiency Virus type 1 and Human cytomegalovirus replication. Since epileptic patients undergoing sodium valproate therapy may suffer from various virus infections, the effect of this drug on replication of viruses especially those affecting neuronal tissues such as Herpes simplex virus type 1 is worthy of investigation.

Methods: Viral replication was studied by quantal response method. Electron microscopy was also performed on cell monolayers treated with the drug and infected with the virus.

Results: Significant reduction in viral infectivity was observed in cell cultures exposed to 0.5-2 mM of sodium valproate either one hr before or after infection. The corresponding electron microscopic examination revealed a very few intracytoplasmic enveloped virions and a marked reduction in the number of intracytoplasmic nucleocapsids in drug-treated virus infected cells compared with those of infected control. However, significant stimulation of virus replication was found upon treatment of cells with 1 mM ($p < 0.01$) and 2 mM ($p < 0.001$) of the drug 24 hours prior to infection. No remarkable change was seen in corresponding electron micrographs.

Conclusion: Although the ultimate outcome of this study awaits in vivo assessments, possible stimulation of Herpes simplex virus type 1 replication by sodium valproate should be considered by clinicians prescribing this drug.

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Keywords • HSV-1 • Sodium valproate • inhibition • electron microscopy

Introduction

Herpes simplex virus type 1 (HSV-1), a double stranded DNA containing virus, is a member of family *Herpesviridae*, and the causative agent of various human infections such as oral and ocular disease as well as a severe form of sporadic encephalitis, although the majority of infections are asymptomatic. The virus has also a propensity for becoming latent in sensory nerve ganglion after a primary infection.¹

Sodium valproate (SV), the sodium salt of valproic acid (VPA) a branched short-chain fatty acid is an effective anticonvulsant and used extensively for the treatment of various epileptic and seizure disorders.² Other attributes of SV include its partial

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degradation by beta-oxidation in hepatocytes mitochondria,³ inhibition of glutathione reductase,⁴ and enhancement of intracellularly reduced form of glutathione,⁵ production of metabolites such as hydrogen peroxide, highly reactive hydroxyl radicals,⁶ and various oxygenated compounds.³

The stimulation of human cytomegalovirus (HCMV) and the immunodeficiency virus type 1 (HIV-1), Human herpes virus-8 (HHV-8), measles virus and poliovirus type 1 by SV has already been described.⁷⁻¹¹ SV-induced potentiation of anti-herpetic effect of acyclovir in HSV-1 infected cells has also been reported.¹² SV may be prescribed for treating seizure followed by central nervous system involvement with HIV-1.⁸ In these patients other opportunistic viral infections such as HSV-1 could be affected by SV. On the other hand, since SV-therapy in epileptic patients may be concomitant with any viral infections including HSV-1, the study of the effect of SV on HSV-1 replication in cell culture system warranted the present investigation.

In this report we describe, both inhibitory and stimulatory effects of SV on HSV-1 replication in cell culture system, using several time intervals between the drug and viral exposures of HeLa cells. In addition, an investigation by electron microscope of cells treated with SV and infected with HSV-1 was undertaken in order to study the influence of the drug on ultrastructural aspects of HSV-1 replication.

Material and Methods

Cell cultures

HeLa cells were grown in Eagle's minimum essential medium, supplemented with 7% fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin. The maintenance medium (MM) was similar, but with 3% FBS. SV (Sigma, USA) was dissolved in MM and diluted to appropriate concentrations. Cytotoxicity assays were performed using trypan blue exclusion dye test,¹³ in which toxic threshold of SV for HeLa cells was found to be about 4 mM.

Virus stock and titration

HSV-1 was isolated from the lip lesion of a patient and confirmed by neutralization test using guinea pig anti-HSV-1 serum (NIH, USA) and monoclonal (D and G) anti HSV-1 antibodies.¹² Virus titration was performed using cell monolayers in 96 microwell plates for 48 hrs under 5% CO₂ at 36°C. The TCID₅₀ was determined using the method of Kärber.¹⁴

SV treatment

Different doses of SV, ranging from 2, 1, 0.5, 0.25 and 0.125 mM were prepared in MM. Cell monolayers were exposed to SV for one and 24 hrs prior to infection, one and 24 hrs after infection, and simultaneous with virus. Appropriate mixtures of SV concentrations and virus (100TCID₅₀) were also incubated for one hr at room temperature before adding to cell monolayers. Following the incubation period, the contents of each series of wells were pooled and stored at -70°C along with their corresponding controls for subsequent infectivity titration. Controls for each series of experiment included uninfected cell monolayers treated with different concentrations of SV, normal cell monolayers and monolayers without exposure to SV and inoculated with the virus. Experiments were performed at least twice in quadruplicates.

Electron microscopy (EM)

HeLa cells grown for 48 hrs in sterile disposable plates with diameter of six cm were treated with 1mM of SV one hr before, one hr after and simultaneously infected with 100 TCID₅₀ of HSV-1. The monolayers were incubated for 24 hrs under 5% CO₂ at 36°C and fixed in 2.5% glutaraldehyde. Processing for EM was performed according to the method described by Robards and Wilson.¹⁵ The sections of SV-treated and untreated control cells were stained with uranyl acetate and lead citrate prior to examination in a Philips TEM, C. M. 10 EM. Each electron micrograph was representative of several cells examined.

Statistical analyses

The statistical analyses were conducted, using analysis of variances and Dunnett *t* test for comparison of test results with those of controls. Values for Mean± SEM are presented in Table 1

Results

The concentrations of SV causing significant stimulation or inhibition of HSV-1 replication are shown in Table 1. The stimulatory or inhibitory effects are dependent on the sequential exposure of cells to SV and virus. As shown in Table1 (No.1-1 to 4-3), the treatment of cells with SV one hr after viral infection (No. 2-2 to 2-4) induced a more significant inhibition of viral replication than that caused by exposing monolayers with similar concentrations of the drug one hr before virus inoculation (No. 1-2 to 1-4) or cells simultaneously treated with SV and virus (No. 3-2 to 3-4). The stimulation of viral replication was found only when

monolayers were treated with 1 and 2 mM of SV 24-hrs before virus inoculation (No. 4-2 to 4-3). The virus replication was not affected by incubation of different SV concentrations and virus at room temperature for one hr prior to inoculation of cell monolayers.

Table1: Effects of incubation of virus with different concentrations (mM) of sodium valproate (SV) causing significant alterations on replication of HSV-1 in HeLa cells.

No	Treatment	Virus yield	P	Effect
1-1	UIC-1	4.650±0.350	-	N
1-2	0.50	1.600±0.659	0.002	I
1-3	2.00	2.050±0.838	0.007	I
1-4	1.00	1.400±0.575	0.001	I
2-1	UIC-2	5.687±0.006	-	N
2-2	0.25	3.750±0.158	0.004	I
2-4	0.50	2.600±0.292	0.000	I
2-3	1.00	1.600±0.540	0.000	I
2-4	2.00	1.550±0.354	0.000	I
3-1	UIC-3	4.300±0.604	-	N
3-2	0.50	1.950±0.398	0.007	I
3-3	1.00	1.600±0.655	0.002	I
3-4	2.00	0.900±0.400	0.000	I
4-1	UIC-4	3.650±0.187	-	N
4-2	1.00	4.450±0.005	0.008	S
4-3	2.00	4.800±0.216	0.000	S

Virus yield (Mean±SEM) = logTCID₅₀/100 µl, UIC= untreated infected control; each control corresponds to underlying experiments, B= before and A= after virus inoculation, SI= simultaneous inoculation with virus; of virus with various concentrations of sodium valproate (SV) at room temperature, N= no effect, I= inhibition, S= stimulation

Electron microscopy

As shown in Fig. 2, similar results were found for different experiments regarding inhibitory effect of SV on HSV-1 replication. Compared with virus infected control cell (Fig. 1), a marked reduction of intracytoplasmic nucleocapsids was found in SV- treated infected cell (Fig. 2). Another prominent feature of the latter was the presence of very few intracytoplasmic enveloped virions relative to infected control cell. SV treated- uninfected cells were similar to untreated cells except for somehow mitochondrial distortions (not shown).

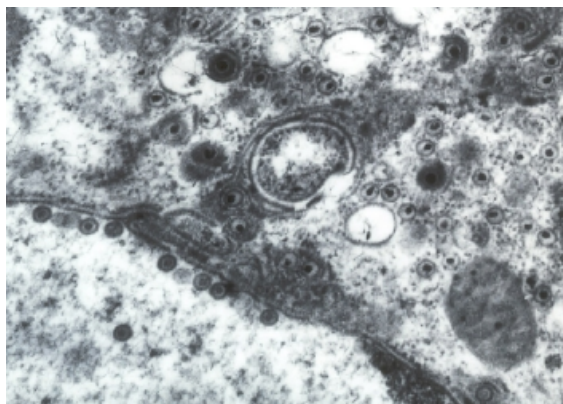


Fig 1: An infected control HeLa cells showing virions in the process of envelopment in perinuclear area and several nucleocapsids within the cytoplasm (X28500).

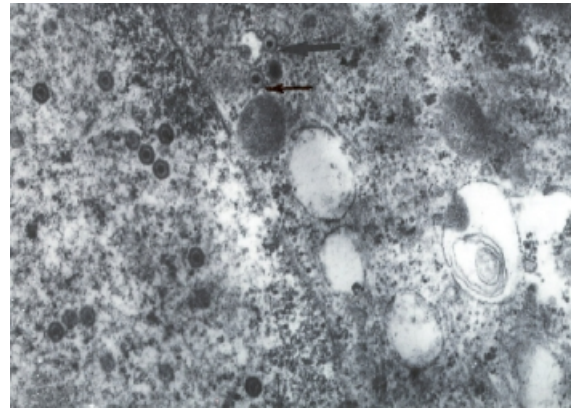


Fig 2: A cell treated with 1mM SV one hr before infection with HSV-1. Note few intracytoplasmic nucleocapsids shown by the arrows. (X28500).

Discussion

The stimulatory effect of SV on viral replication has been reported for HCMV, HIV-1, HHV-8, measles virus and poliovirus type 1.⁷⁻¹¹ The enhancing together with the inhibitory effects of SV on HSV-1 replication is described in the present report. The treatment of cells with SV one hr before and after virus infection and also simultaneous exposure of cells to both SV and virus all caused inhibition of viral replication. (Table 1; No. 1-1 to 3-4) This inhibitory effect was probably due to the interaction of SV metabolites, namely highly reactive hydroxyl radicals and hydrogen peroxide (H₂O₂) with the synthesis of viral macromolecules.⁶

To clarify whether the inhibitory effect was due to direct inactivation of virus by SV, various concentrations of the drug were incubated with the virus at room temperature for one hr before inoculation of the cell cultures. This experiment ruled out any such virucidal effect. In this context, it was puzzling to find that various drug concentrations had no significant influence upon virus yield. It was thus tempting to speculate that pre-incubation of SV and virus at room temperature induced an irreversible binding of SV to viral envelope. SV remained attached to the cell membrane upon and subsequent fusion of viral envelope with plasma membrane; it could not be released into the cell.

An interesting observation was the stimulation of viral replication by 1 and 2 mM of SV applied to cells 24 hrs before infection with the virus, (Table 1; No 4-2, 4-3). Kuntz-Simon at al. reported a similar effect on HCMV grown in MRC5 cells and found it very difficult to propose a hypothesis explaining the stimulation of HCMV promoter and replication.⁷ They suggested that this effect was probably due to modification of intracellular redox balance, which regulated activation of various transcription factors

that involved the stimulation of HCMV replication.⁷ This event could probably induce higher cellular H₂O₂ and decreased glutathione reductase that produced a reduced form of glutathione (GSH), a major antioxidant.⁴ A similar mechanism might be operative in stimulation of HSV-1 replication by SV. Similar to our findings regarding the stimulation of HSV-1 replication by SV, in previously reported enhancement of HCMV,⁷ the corresponding cell cultures were treated with SV only 24 hours before virus inoculation. However, the inhibition of HCMV replication by SV might have occurred if exposure to the drug were extended to other time intervals, such as those performed in present investigation.

In another study, no stimulatory effect of 0.5 and 1 mM of SV were observed when cells were exposed to SV 24 hr before HSV-1 infection. Such a difference could be due to different experimental conditions including moi of HSV-1, as well as reporting a slight and insignificant HSV-1 replication with 2 and 4 mM of SV.¹²

In our opinion, a series of time-dependent intracellular reactions requiring critical concentrations of SV may be involved in enhanced HSV-1 replication. These events could probably induce higher cellular hydrogen peroxide (H₂O₂) and decreased glutathione (GSH), a major antioxidant.³⁻⁵

It is noteworthy to point out that other investigations reporting the in vitro enhancement of virus replication by SV allowed only 24 hrs interval between drug exposure and viral infection.^{8,12}

In our studies the treatment of cells with the drug 24 hours after infection with HSV-1 had no significant effect on virus yield. The rationale for this observation might be that the synthesis of viral macromolecules proceeded unabated in the absence of SV.

Electron microscopic examination of SV-treated infected cells supported our finding with regard to the inhibitory effect of the drug on viral replication. This study revealed a very few intracytoplasmic enveloped virions and a marked reduction in the number of nucleocapsids within the cytoplasm of SV-treated virus infected compared with infected control cells. This triggers the speculation that intracytoplasmic disruption of nucleocapsids and impairment of viral envelopment might occur in SV-treated infected cells.

Either stimulation of HCMV and HIV-1 or both stimulatory and inhibitory effect of SV on HSV-1 replication under in vitro conditions can not be substantiated until such experiments were extended to appropriate in vivo assessments. Until then and in the event of HSV-1

infection in patients undergoing SV- therapy it will be prudent to consider its probable stimulatory effect on viral replication and if possible seeking other substitutes. In this context, other investigators have mentioned possible risk of SV therapy in patients infected with HIV-1,^{16, 17} and measles virus.¹⁸

Conclusion

Although the ultimate outcome of this study awaits in vivo assessments, possible stimulation of Herpes simplex virus type 1 replication by SV should be considered by clinicians prescribing this drug.

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