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Efficacy of sodium polyanethol sulfonate on herpes simplex virus-1 infection in vitro

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Objective: To investigate the effect of sodium polyanethol sulfonate (SPS) on herpes simplex virus type 1 (HSV-1) infection in vitro.

Methods: Human corneal epithelial (HCE-T) cells and Vero cells were infected with HSV-1 [HSV-1 f strain, HSV-1f; HSV-1-H129 with green fluorescent protein (GFP) knock-in, HSV-1g]. SPS was added to the culture medium at various concentrations in time-of-addition assay. Experiments including photography of fluorescence in HSV-1g or plaque formation by HSV-1f, western blot assays, real-time RT–PCR assays, cytopathic effect inhibition assays, cytotoxicity assays, and viral absorption and penetration assays were performed to explore the antiviral effect and mechanism of the compounds.

Results: We identified that SPS reduced the replication of HSV-1 in HCE-T and Vero cells in a dose-dependent manner. HSV-1g fluorescence was reduced by 66.3% and 65.4% in HCE-T and Vero cells, respectively, after treatment with 0.4 μg/ml SPS. Furthermore, the viral fluorescence intensities were inhibited by SPS in a dose-dependent manner when the viruses or cells were preincubated with SPS. Relative levels of the ICP4 protein and VP16 mRNA were decreased by SPS in a dose-dependent manner. Moreover, the IC_{50} values of SPS for HSV-1g and HSV-1f in HCE-T cells were 0.69±0.09 μg/ml and 1.63±0.44 μg/ml, respectively. Even 10,000 μg/ml SPS had no obvious cytotoxicity toward HCE-T and Vero cells. Importantly, viral absorption and penetration assays showed that the relative fluorescence intensity of HSV-1g was significantly reduced by SPS in a dose-dependent manner in the absorption test, but no change was observed in the penetration test.

Conclusions: SPS inhibits HSV-1 replication in HCE-T and Vero cells, indicating that SPS has the potential for treating HSV-1 infection, particularly HSV-1 keratitis.

Herpes simplex virus type 1 (HSV-1) infection is extremely common in humans, and approximately 50% and 75% of the adult populations in the United States [1] and Germany [2], respectively, have been infected with this virus. HSV-1 may cause encephalitis, cutaneous herpes, keratitis, conjunctivitis, uveitis, and other conditions. Ocular HSV-1 infection can result in serious visual impairment or even blindness. Epithelial keratitis is the most common type of ocular HSV-1 infection, with over 50% of patients presenting with corneal epithelial lesions. Primary ocular infections tend to appear in youths, and after primary infections are cleared, the virus hides in the trigeminal ganglia or cornea, where it maintains a state of latency. When stimulation occurs, such as weakened immunity, corneal injuries, or ultraviolet exposure, latent viruses are activated, producing many infectious virus particles that result in recurrent keratitis.

Although HSV-1 infection is prevalent worldwide, no effective vaccine has been widely used [3]. Antiviral drugs currently face challenges. The overuse of nucleoside analogs, such as acyclovir (ACV), which suppresses viral DNA polymerase, has led to drug resistance. HSV resistance to ACV is usually isolated in immunocompromised patients, and its incidence ranges from 3.5% to 10% [4]. These circumstances have prompted researchers to prioritize the identification of new antiherpetic drugs and their new targets. Inhibitors of absorption, mainly heparan sulfate, sulfated oligosaccharides [5], and sulfated polysaccharide [6], have been reported for several decades. These negatively charged molecules interact with the positive charges on the viral glycoprotein or on cell surface receptors, thus preventing the absorption of the virus into cells [6].

Sodium polyanethol sulfonate (SPS) is a polymer with a high molecular weight of 9–11 kDa. As an anticoagulant, it is widely used for the bacterial culture of blood samples from patients [7]. Although no report has described the interrelation of SPS and viruses, derivatives of SPS, such as poly(4-styrenesulfonic acid-co-maleic acid; PSM) [8], PRO 2000 [9], and poly(sodium 4-styrenesulfonate; PSSNa) [10], have shown their inhibitory activity against HSV-1 replication. Here, we investigated the activity of SPS in inhibiting

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HSV-1 infection in vitro and explored specific mechanisms to identify a new treatment for HSV-1 infection.

METHODS

Reagents, cells, viruses: SPS was purchased from Sigma–Aldrich (St. Louis, MO). HCE-T cells (human corneal epithelial cells-transformed, RCB2280, RIKEN Cell Bank, Japan) were cultured in DMEM/F-12 (Gibco, Grand Island, NY) supplemented with 5 μg/ml insulin (Sigma–Aldrich), 10 ng/ml epidermal growth factor (Sigma–Aldrich), 1% penicillin and streptomycin (Gibco), and 10% fetal bovine serum (FBS, Gibco) at 37°C with 5% CO_2. Vero cells (African green monkey kidney cells, ATCC, CCL-81) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) containing 5% fetal bovine serum (Gibco) and 1% penicillin and streptomycin (Gibco) at 37°C in the presence of 5% CO_2. Two strains of HSV-1, the HSV-1 f strain (HSV-1f) [11] and the GFP-expressing HSV-1 strain [12] (HSV-1 strain-H129 with GFP knock-in, HSV-1g, which was a gift from Professor Minhua Luo, Wuhan Institute of Virology, Chinese Academy of Sciences), were used in this study.

Time-of-addition assay: Time-of-addition assays were conducted to explore which stage of the HSV-1 lifecycle SPS exerts an obvious effect. HCE-T and Vero cells were seeded in 12-well plates. Cells became confluent after 1–2 days and then were infected with HSV-1g at a multiplicity of infection (MOI) of 0.1 under the following three conditions: (i) preincubation of cells with SPS: HCE-T cells were preincubated with different concentrations of SPS (0, 1, 5, or 10 μg/ml) for 1 h at 37°C and then either washed or not washed three times with PBS before infection with HSV-1g; (ii) preincubation of viruses with SPS: HSV-1g was cocultured with different concentrations of SPS (0.05, 0.1, or 0.2 μg/ml) at 37°C for 1 h before being used to infect HCE-T cells for 1 h. The mixture culture medium was subsequently removed, and HCE-T cells were cultured with a basal culture medium; and (iii) postinfection: After the infection of HCE-T cells and Vero cells with HSV-1g for 1 h, different concentrations of SPS (0, 0.1, 0.2, or 0.4 μg/ml) were added to the basal culture medium. The process of virus infection was conducted as described in our previous report [13]. Briefly, after removing the culture medium, 200 μl of virus inoculum in the medium was added to the cells with shaking every 15 min at 37°C for 1 h. The viral inoculum was then removed by three washing steps, and the infected cells were cultured in DMEM without fetal bovine serum. At 24 h postinfection, a fluorescence microscope with a digital camera (Nikon Eclipse TS100F, Japan) was used to observe and photograph cultured cells. The fluorescence intensity was measured using ImageJ software (NIH, Bethesda, MD).

Viral plaque assay: The antiviral activity of SPS was also evaluated by plaque assay [14]. The HCE-T cells were seeded into each well of a 12-well plate and reached nearly 100% confluence. Before viral infection, the culture medium was removed and washed with PBS, and the cells were incubated with HSV-1f inocula (50–100 PFU/well) for 1 h at 37°C. Next, the inocula were removed, the HCE-T cells were washed with PBS and then overlaid with 1 mL medium containing 0.5% methylcellulose, 2% FBS, 1% penicillin and streptomycin, and various levels of SPS (0.3125, 0.625, 1.25, 2.5, 5, or 10 μg/ml). After 48 h of incubation, the HCE-T cells were fixed with 4% formaldehyde and stained with 0.5% crystal violet. Plaque-forming units (PFU) were counted.

Western blot assay: The HCE-T cells were seeded on a 6-well plate and then infected with HSV-1f at an MOI of 0.1 for 1 h. Residual viruses were removed by washing with PBS, and the cells were exposed to different concentrations of SPS (0.625, 1.25, or 2.5 μg/ml) for 24 h. Next, the cells were lysed with RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) on ice. Cell lysates were centrifuged at 12,000 × *g* for 10 min at 4°C. The protein concentrations in the supernatants were quantified using the bicinchoninic acid (BCA) assay (Beyotime Biotechnology). Western blotting was performed as previously described [15]. Briefly, 15 μg of protein was separated using 4%–12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Merck Millipore, Billerica, MA) that was then blocked with bovine serum albumin (BSA). The PVDF membrane was incubated overnight at 4°C with a mouse monoclonal antibody against the ICP4 protein (1:800 dilution, Abcam Ltd., Cambridge, UK) or a rabbit monoclonal antibody against the β-actin protein (1:1000 dilution, Cell Signaling Technology, MA). After incubation with a secondary antibody at room temperature for 1 h, the membrane was detected with an Immobilon Western chemiluminescence HRP Substrate (Merck Millipore Corp.). The density of the proteins was determined using ImageJ software (NIH).

Real-time RT–PCR assay: The HCE-T cells were infected with HSV-1f at an MOI of 0.1 for 1 h and then exposed to different concentrations of SPS (0.625, 1.25, or 2.5 μg/ml) for 24 h. Total RNA was extracted using an RNA extraction kit (Tiangen, Beijing, China). RT–PCR was performed using the One Step TB Green Prime Script RT–PCR Kit (Takara, Kusatsu, Japan). The primer sequences were as follows: GAPDH mRNA, forward 5′-GCACCGTCAAGGCTGAGAAC-3′ and reverse 5′-TGGTGAAGACGCCAGTGGA-3′; and VP16

mRNA, forward 5′-TTTGACCCGCGAGATCCTAT-3′ and reverse 5′-GCTCCGTTGACGAACATGAA-3′. RT–PCR was performed at 42°C for 5 min, 95°C for 10 s, and 40 cycles of 95°C for 5 s, 55°C for 30 s, and 72°C for 30 s using the instrument (LightCycler 480, Roche). The relative CT values of the HSV VP16 mRNA were measured with the comparative method ($2^{-\Delta\Delta CT}$) using data from triplicate experiments [16].

Cytopathic effect inhibition assay: The 50% inhibitory concentration (IC_{50}) was determined by performing a cytopathic effect (CPE) inhibition assay using a previously reported method, with slight modifications [17]. The HCE-T cells were seeded on a 96-well plate, followed by infection with HSV-1f or HSV-1g at an MOI of 0.1 for 1 h. The cells were then exposed to different concentrations of SPS (0.3125, 0.625, 1.25, 2.5, 5, or 10 µg/ml). At 24 h postinfection, the cells were dyed with neutral red for 3 h at 37°C. After the neutral red was removed, lysis buffer was added to release intracellular neutral red and incubated with shaking for 10 min. The intensity of neutral red was measured using an ELISA plate reader (BioTek Synergy, VT) at 540 nm. The inhibition rate was determined as follows [18]:

$$[(OD_T)_{HSV}\text{-}(ODc)_{HSV}]/[(ODc)_{MOCK}\text{-}(ODc)_{HSV}]\times 100\ (\%),$$

where $(OD_T)_{HSV}$ indicates the optical density of the test sample (containing a compound), $(ODc)_{HSV}$ indicates the optical density of the virus-infected control (no compound), and $(ODc)_{MOCK}$ indicates the optical density of the mock-infected control (no virus or compound).

Cytotoxicity assay: The effects of SPS on the viability of HCE-T and Vero cells were determined by conducting a cell counting kit-8 assay, as previously reported [19]. Cells were dispensed into 96-well plates, and when the cells reached 90% confluence, various concentrations of SPS (1, 10, 100, 1,000, or 10,000 µg/ml) were added to the media. After 24 h or 48 h, the cells were washed with PBS and cultured with 100 µl of fresh medium. Then, 10 µl of CCK-8 reagent (CCK-8, Dojindo, Kumamoto, Japan) was added to each well, and the cells were incubated for another 2 h. The optical density value was measured using an enzyme-labeled instrument at 450 nm. Cell viability was determined as follows:

$$\text{Cell viability}=[OD_{\text{SPS 1–10000 µg/ml}}\text{-}OD_{control}]/[OD_{\text{SPS 0 µg/ml}}\text{-}OD_{control}]\times 100\ (\%).$$

Viral absorption and penetration assays: Viral absorption and penetration assays were performed as previously reported, with modifications [9,20]. HCE-T cells were seeded in 12-well plates and grown until 90% confluence was reached. For the absorption assay, after precooling at 4°C for 1 h, the cells were absorbed with HSV-1g (MOI 0.1) in the presence of SPS (0.05–0.2 µg/ml) at 4°C for 2 h. Then, by washing with PBS to remove the unabsorbed virus, the infected cells were overlaid with culture medium and transferred to 37°C. After 24 h of incubation, each well was observed by fluorescence photography.

For the penetration assay, the HCE-T cells growing in 12-well culture plates were precooled at 4°C for 1 h, followed by infection with HSV-1g (MOI 0.1) for 2 h at 4°C. After removing the inocula and washing with PBS, the cells were incubated with SPS (0.1–0.4 µg/ml) for 1 h at 37°C to allow penetration. The cells were then washed with citrate buffer (pH 3.0) to inactivate viruses on the cell surface and washed with PBS three times. The culture medium was added and cultured for 24 h. The fluorescence intensity was measured as described above.

Statistical analysis: Each experiment was conducted at least three times. GraphPad Prism software 5 was used to create the charts. The results were evaluated using a one-way ANOVA (ANOVA). A p-value < .05 was considered statistically significant.

RESULTS

SPS reduced the replication of HSV-1 in HCE-T and Vero cells: HSV-1-infected HCE-T and Vero cells were exposed to different concentrations of SPS (0, 0.1, 0.2, of 0.4 µg/ml) to investigate the antiviral activity of SPS in cells. After 24 h of incubation, the antiviral effect on HSV-1 was evaluated by measuring the GFP fluorescence of HSV-1g (Figure 1A,B). Decreased fluorescence intensities were observed in both HCE-T and Vero cells treated with increasing concentrations of SPS. At a concentration of 0.4 µg/ml SPS, the HSV-1g fluorescence was reduced to 33.7% and 34.6% in HCE-T and Vero cells, respectively (Figure 1C,D). In the plaque assay, PFU was reduced in a dose-dependent manner in HCE-T cells infected with HSV-1f when exposed to SPS. At a concentration of 10 µg/ml SPS, the percentage of PFU reached 2.8% (Figure 1E,F).

Effects of time-of-addition on SPS-mediated inhibition of HSV replication: The effects of SPS on different stages of the HSV-1 lifecycle were measured by performing a time-of-addition assay. SPS-treated cells (Figure 2) or SPS-treated HSV-1g (Figure 3) were used to evaluate the GFP fluorescence of HSV-1g in HCE-T cells at 24 h postinfection. In SPS-preincubated cells with or without PBS washing, the relative fluorescence intensity did not show significant differences between cells treated with 1 µg/ml SPS that were or were not subsequently washed. However, when the concentration of SPS reached 5 µg/ml or 10 µg/ml, the antiviral activity of SPS under the no-washing condition was much higher than that of the washing condition (Figure 2).

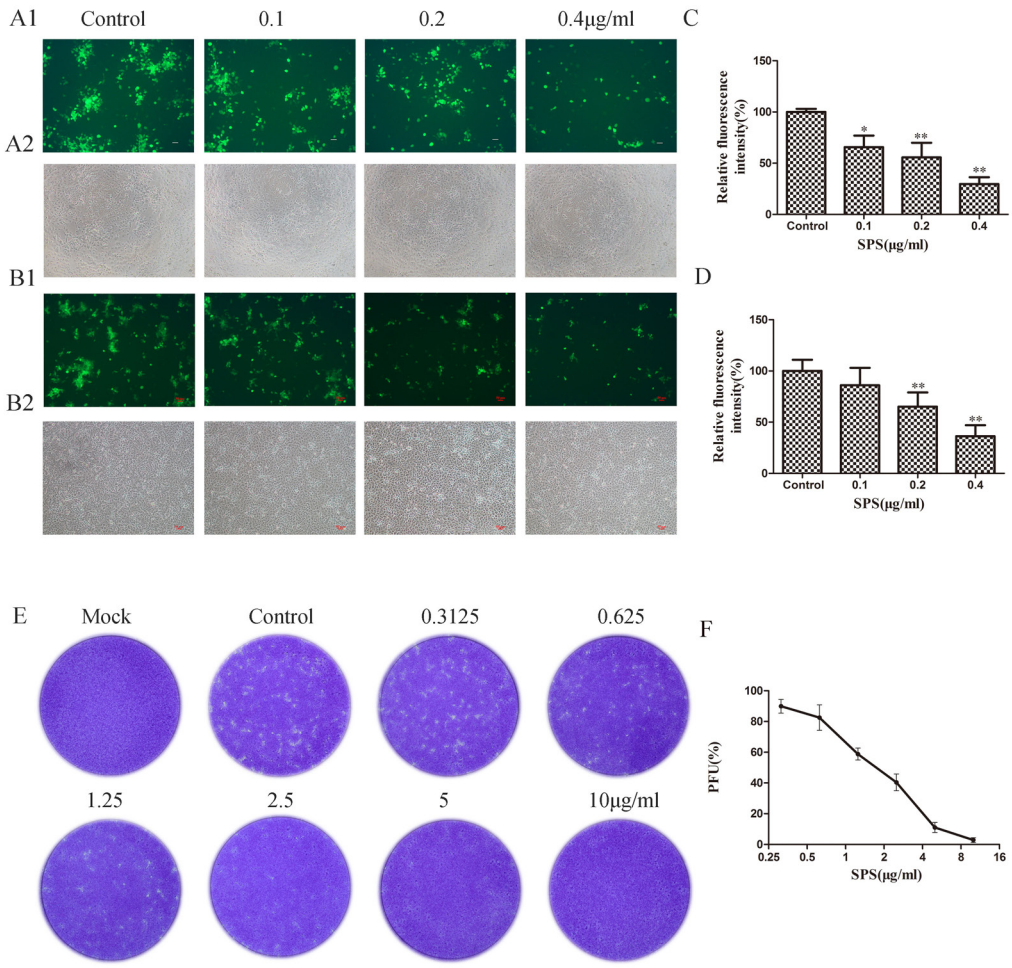

Figure 1. The inhibitory effect of SPS on HSV-1 in HCE-T and Vero cells. HCE-T (A, C) and Vero (B, D) cells were incubated with HSV-1g for 1 h at 37°C and then cultured with different concentrations of SPS for 24 h. The control groups included cells treated without SPS. Phase contrast photos (A2, B2) show the same fields of cells as fluorescent photos (A1, B1). GFP fluorescence in A and B was quantified using ImageJ software (C, D). The average intensity of the control cells was assigned a value of 100. Viral plaque assay (E) was conducted in HCE-T cells. The cells were incubated with HSV-1f for 1 h at 37°C and then exposed to SPS for 48 h. Mock groups refer to uninfected cells treated without SPS. The percentages of PFU from E were calculated (F; versus the control group). Data are presented as the means ± standard deviations (n = 3). *$p < .05$ and **$p < .01$ (compared with the control group).

When the antiviral effect of SPS was evaluated by treating HSV-1 with SPS before the infection of HCE-T cells, the viral fluorescence intensity was reduced by 91% in the presence of an SPS concentration of 0.05 μg/ml (Figure 3). In addition, more remarkable inhibition of viral fluorescence was observed in the 0.1 and 0.2 μg/ml SPS-treated samples.

SPS reduced ICP4 protein and VP16 mRNA levels: The antiviral effect of SPS on HSV-1f was measured by quantifying the ICP4 protein and VP16 mRNA in HCE-T cells. SPS (0.625, 1.25, or 2.5 μg/ml) was immediately administered 1 h after the viral infection, followed by incubation for another 24 h. As shown in Figure 4, the western blot analysis showed that SPS decreased the relative levels of the ICP4 protein in a dose-dependent manner. At a concentration of 2.5 μg/ml, SPS reduced the relative levels of the ICP4 protein by 63% (Figure 4A,B). Based on the results of the RT–qPCR assay, a significant dose-dependent reduction in the expression of VP16 mRNA was also observed (Figure 4C). At a concentration of 2.5 μg/ml, SPS decreased the relative levels of VP16 mRNA by 65%.

Half maximal inhibitory concentration of SPS: A CPE inhibition assay was conducted to calculate the IC_{50} of SPS toward

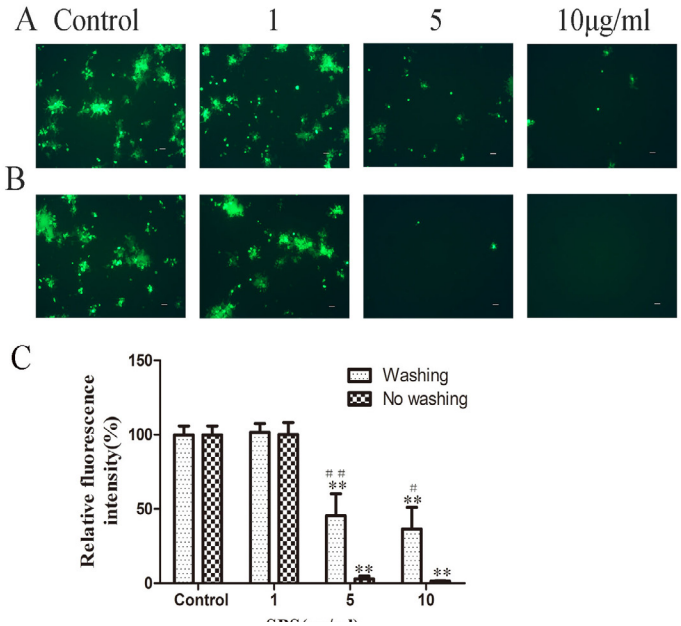

Figure 2. The antiviral effect of SPS on preincubated HCE-T cells. HCE-T cells were preincubated with various concentrations of SPS for 1 h at 37°C and then subjected to washing (A) or no washing (B) with PBS before inoculation with HSV-1g. After 24 h, GFP fluorescence was detected using fluorescence microscopy and quantified using ImageJ software (C). The average intensity of the control cells was assigned a value of 100. Values are presented as means ± SD (n = 3). ***p* < .01 compared with the control group.

HSV-1g and HSV-1f and to confirm the effective concentrations of SPS against HSV-1. As shown in Figure 5, the IC_{50} values of SPS for HSV-1g and HSV-1f in HCE-T cells were 0.69±0.09 μg/ml and 1.63±0.44 μg/ml, respectively. At a concentration of 10 μg/ml SPS, the maximum inhibition rates of HSV-1g and HSV-1f were 95% and 91%, respectively.

Cytotoxicity of SPS to HCE-T and Vero cells: The cytotoxicity of SPS was tested using a cell proliferation assay. When the HCE-T and Vero cells were exposed to different

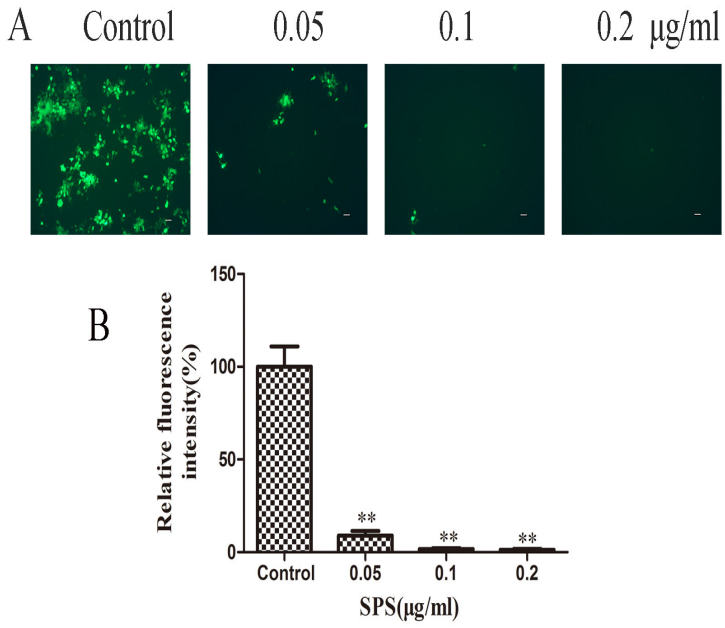

Figure 3. The antiviral effect of SPS on HSV-1 after preincubation. HSV-1g was preincubated with SPS for 1 h at 37°C, and the mixture was then used to infect HCE-T cells for 1 h at 37°C, followed by washing and culturing in a base medium for another 24 h. (A) HSV-1g replication was detected using fluorescence microscopy. (B) The average fluorescence intensity was measured using ImageJ software. The average intensity of the virus control was assigned a value of 100. Data are presented as the means ± standard deviations (n = 3). ***p* < .01 compared with the control group.

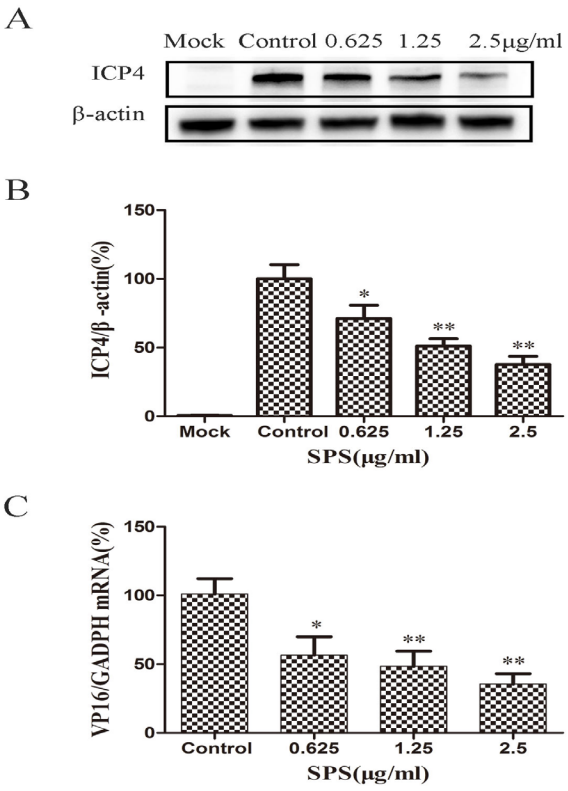

Figure 4. SPS decreased the expression of the ICP4 protein and VP16 mRNA. (A) HCE-T cells were preincubated with various concentrations of SPS followed by infection with HSV-1f, and ICP4 and β-actin levels were then measured using western blotting. (B) Average gray value intensities of western blot bands were measured using ImageJ software. Uninfected cells were set as a mock group, and infected cells without SPS preincubation were set as the control group. The intensity of the control group was assigned a value of 1 (n = 3). $^{**}p < .01$. (C) The VP16/GAPDH ratio in cells treated with different concentrations of SPS was determined by RT–PCR. Data are presented as means ± standard deviations (n = 3). $^{**}p < .01$ compared with the control group.

concentrations of SPS (1, 10, 100, 1,000, or 10,000 μg/ml) for 24 h or 48 h, the OD values of CCK-8 staining were detected to reveal the cell viabilities. The viability of the HCE-T cells fluctuated within the 10% range as the SPS levels increased from 1 to 10,000 μg/ml at both 24 and 48 h (Figure 6A). The viability of the Vero cells showed an increasing trend when SPS levels increased from 1 to 1000 μg/ml (Figure 6B). At an SPS concentration of 10,000 μg/ml, the viability of the Vero cells increased by 12% and 43% after 24 h and 48 h of incubation, respectively, compared to 1 μg/ml. No reduction in cell viability was observed under our various conditions, even when the cells were incubated with 10,000 μg/ml SPS for 48 h.

SPS interferes with the HSV-1 absorption process: The effects of SPS on the early stages of the HSV-1 lifecycle were further measured by conducting absorption and penetration assays. SPS was administered during incubation at 4°C (absorption) or at the time that the cells were transferred to 37°C (penetration). The antiviral effect on HSV-1 was measured by detecting the GFP fluorescence of HSV-1g (Figure 7). When SPS was added at 4°C for the absorption test, the relative fluorescence intensity of HSV-1g was significantly reduced by SPS in a dose-dependent manner. At a concentration of 0.1 μg/ml SPS, the relative fluorescence intensity of HSV-1g decreased by 94% compared with that of the control cells (Figure 7A,C). On the other hand, when SPS was added after recovery to 37°C for the penetration test, no significant alteration in GFP fluorescence occurred, even when SPS levels increased from 0.1 to 0.4 μg/ml (Figure 7B,D).

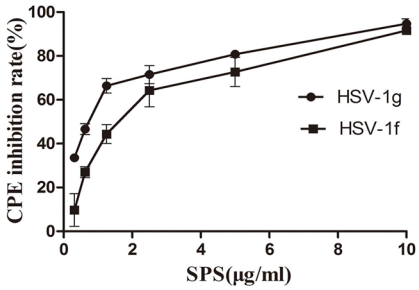

Figure 5. Half maximal inhibitory concentration of SPS for HSV-1f and HSV-1g. The CPE inhibition of HSV-1f and HSV-1g was tested using neutral red kits. All results are presented as a percentage of the control group. Data are presented as means ± standard deviations (n = 9).

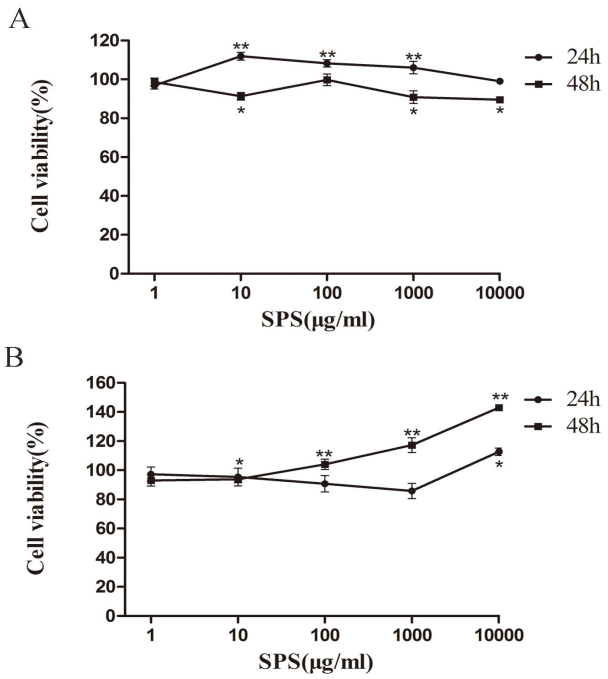

Figure 6. Cell viability in the presence of various SPS concentrations. (A) HCE-T and (B) Vero cells were exposed to SPS for 24 or 48 h, and cell viability was determined using the CCK-8 colorimetric assay. All results are presented as a percentage of the control group (0 μg/ml SPS). Data are presented as means ± standard deviations (n = 6).

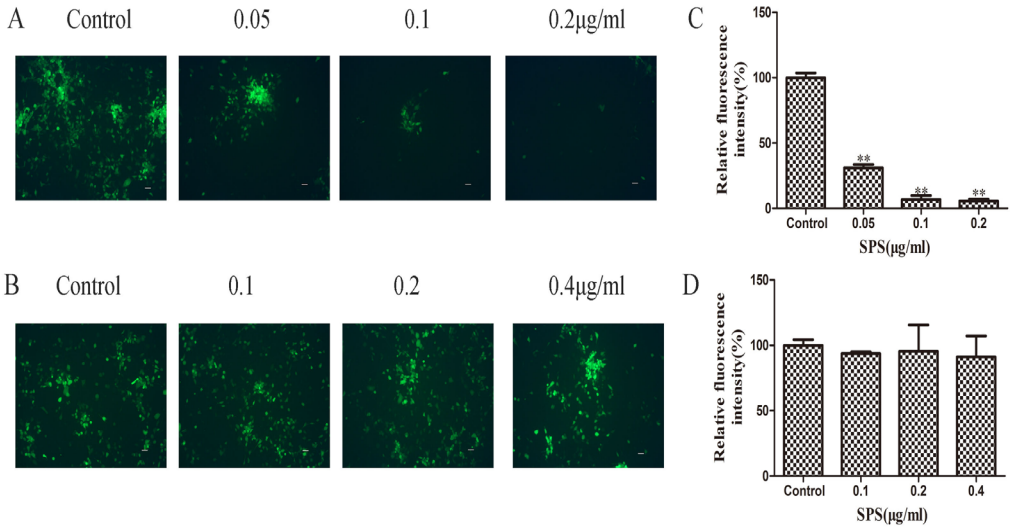

Figure 7. SPS inhibited HSV-1 infection by blocking viral absorption. HCE-T cells were incubated with HSV-1g for 2 h at 4°C, and different concentrations of SPS were then added during absorption (A) and penetration (B). GFP fluorescence during absorption (C) and penetration (D) was detected using an inverted fluorescence microscope. The average fluorescence intensity was measured using ImageJ software. The average intensity of the control cells was assigned a value of 100. Data are presented as means ± standard deviations (n = 3). ***p* < .01 compared with the control group. **p* < .05 compared with the control group.

DISCUSSION

We have proven the strong antiviral effect of SPS on HSV-1 in HCE-T and Vero cells. Pretreatment of cells or HSV-1 with SPS also induced obvious viral inhibition. SPS showed no cytotoxicity toward either HCE-T or Vero cells in vitro and tended to inhibit the viral absorption process. Our results suggest that SPS is a potential candidate anti-HSV-1 drug treatment. However, we did not investigate the inhibitory effect of SPS in vivo. In addition, the antiviral effects of SPS on other types of viruses require further study.

SPS is a polyanionic compound with multiple biological functions. Previous studies have reported that SPS blocks the activity of complement proteins [21] and neutralizes some antibiotics [22]. No reports have documented the effect of SPS on viruses until now. Polyanionic compounds contain biologic anionic polysaccharides and synthetic sulphonic acid-containing polymers (SPS and its derivatives) [23-25]. Several sulfated polysaccharides (dextran sulfate, pentosan polysulfate, fucoidan, and carrageenans) have been proven to be potent and selective inhibitors for various enveloped viruses [26]. Derivatives of SPS have also recently shown antiviral activity. In 2012, Min et al. reported that PSM inhibits both HIV and HSV-1 infections in vitro and that HSV-2 vaginal infection was controlled by PSM gels in mice [8]. Recently, poly(sodium 4-styrenesulfonate) was demonstrated to inhibit Zika virus replication in vitro. It acts mostly through binding to the Zika virus and blocking viral absorption [10]. We incidentally found that SPS had inhibitory effects on HSV-1 replication and conducted the present study. Furthermore, SPS has been applied as a drug to treat kidney failure in animals. Glomerular fibrinolytic activity in rats was remarkably increased by an intravenous injection of SPS [27]. Spencer and colleagues reported that an intravenous injection of SPS successfully treated acute kidney failure in 38 puppies, with no animals dying due to the toxicity of SPS [28]. Based on these studies, SPS has little or no toxicity in vivo and has the potential for topical use in the eyes. Ocular HSV-1 infection frequently occurs on the ocular surface, manifesting as conjunctivitis and keratitis. Further in vivo studies are needed to determine whether SPS can be topically used to treat HSV-1 infection.

Polyanions have been reported to possess antiviral activity that is attributed mainly to the inhibition of viral binding to cells and virus–cell fusion [29]. These polyanionic compounds may interact with the positive charges present on the virus or on the cell surface and inhibit virus adsorption to the host cells. Marine sulfated polysaccharides have been proven to prevent SARS-CoV-2 host cell entry, and the S glycoprotein, which is located on the envelope of SARS-CoV-2, has been shown to be the most likely target of sulfated polysaccharides [30]. Regarding herpesvirus, HSV-1 is covered by an envelope with at least 12 different glycoproteins [31]. Glycoproteins, such as gB, gC, gD, and gH/L, are related to viral attachment to host cells [32]. Our data showed that SPS inhibits HSV-1g replication by blocking absorption rather than penetration of the virus. However, we could not exclude the possibility that SPS directly bind to viruses in our model, in addition to blocking virus absorption. It likely acts on the glycoproteins of HSV-1, although this remains to be confirmed.

In conclusion, SPS inhibits HSV-1 infection in HCE-T and Vero cells. Furthermore, SPS reduces the replication of HSV-1 in HCE-T cells after the preincubation of cells and viruses, and postinfection. In addition, SPS had no toxic effect on the growth of HCE-T and Vero cells. The most likely mechanism of inhibition of HSV-1 was as an entry inhibitor that prevented HSV-1 attachment to the target cells. Our data suggest that SPS represents a potential drug for HSV-1 infection.

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