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Inhibition of herpes simplex virus infection by oligomeric stilbenoids through ROS generation

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ABSTRACT

Stilbenoids including resveratrol contain the basic structural unit of 1,2-diphenylethylene. Naturally occurring stilbenoids have broad structural features due to oligomerization and modifications and some have demonstrated potent biological activities. In an effort to identify bioactive stilbenoids, we screened a group of dimeric and oligomeric stilbenoids against HSV-1 and HSV-2 infection. Several trimeric and tetrameric derivatives showed anti-herpetic activity at single digit micromolar concentrations. HSV-1 and HSV-2 replication requires for NF-κB and MAPK activation. The compounds showed no inhibitory activity against NF-κB and Erk/MAPK activation, instead those compounds promoted rapid and transient release of reactive oxygen species (ROS). Addition of *N*-acetylcysteine (NAC), a scavenger of ROS, reversed the inhibitory effect of those compounds against HSV replication. In addition to the identification of resveratrol derivatives with potent anti-HSV activity, our results uncover a mechanism of polyphenol-mediated anti-HSV response, linking anti-herpetic activity of oligomeric stilbenoids to innate immunity.

1. Introduction

Resveratrol is a natural compound originally isolated from *Veratrum grandiflorum* by Takaoka (1939) and the compound is widely distributed in plants of several families. Resveratrol has gained enormous attention due to demonstrated ability to prevent a wide variety of conditions, including cancer, cardiovascular diseases, neurodegenerative diseases (Jang et al., 1997; Pervaiz, 2003; Baur and Sinclair, 2006; Athar et al., 2007; Vang et al., 2011), and bacterial and viral infections (Campagna and Rivas, 2010). The compound is active against DNA and RNA viruses, including HIV, influenza viruses, RSV, CMV, EBV, HSV-1 and -2, and SARS, to name a few. The reported mechanisms include suppression of signaling events. But the activity is generally associated with high concentrations applied.

Like resveratrol, oligomeric resveratrol derivatives are also widely distributed in plants or synthetically prepared and some of them have demonstrated biological activity at micromolar concentrations (Shen et al., 2009; Spatafora and Tringali, 2012). In an attempt to identify naturally occurring resveratrol derivatives, we tested a panel of more than 30 dimeric and oligomeric resveratrol derivatives using HSV-1 and HSV-2 infection of Vero cells as model systems. HSV-1 is the most commonly acquired form of herpes and common cause of oral herpes, but approximately 30% of genital herpes can be associated with HSV-1. HSV-2 infection mainly affects the genital area and is a major cause of sexually transmitted disease. Both HSV-1 and HSV-2 can also establish latency in the nervous system. More than 60% of the entire human population experience oral herpes infections, while genital herpes by both HSV-1 and HSV-2 affects approximately 1 billion individuals worldwide (Fleming et al., 1997; CDC, 2010). HSV infection is also a major cause for transmission of STD, including HIV and HPV (Corey, 2007; Nagot et al., 2007; Buve and Lynen, 2010).

We identified several compounds with anti-HSV activity at micromolar concentrations. Unlike resveratrol that blocks HSV infection through inhibition of NF-κB activation, those compounds showed no activity against NF-κB or MAPK activation required for HSV replication. Instead, those compounds promoted rapid but transient release of ROS, important mediators of innate immunity (Valyi-Nagy and Dermody, 2005; Spooner and Yilmaz, 2011). Here we report the characterization of oligomeric resveratrol against HSV infection.

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2. Materials and methods

2.1. Cells and viruses

HeLa cells were purchased from ATCC (Manassas, VA). African green monkey kidney epithelial Vero cells and H1299 cells were purchased from Cell Bank of Chinese Academy Sciences (Shanghai, China). H1299 is a human non-small cell lung carcinoma from the lymph node and does not express p53 protein (Lin and Chang, 1996). Cells were cultured in DMEM with high glucose (Invitrogen) that was supplemented with 10% heat inactivated fetal bovine serum (Invitrogen), 2 mM L-glutamine, nonessential amino acids and sodium pyruvate (Invitrogen), and maintained at 37 °C in a humidified incubator with 5% CO_2.

Herpes simplex virus type 2 strain G was purchased from ATCC (VR-734). Replication-competent herpes simplex virus type 1 (strain 17) with non-necessary gene (*UL2*) replaced by enhanced green fluorescent protein gene (HSV-1/EGFP) was purchased from Beijing FivePlus Molecular Medicine Institute (Beijing, China). The viruses were propagated in Vero cells and virus titer was determined as reported (Ejercito et al., 1968).

2.2. Reagents

Stilbenoids used in this study (Fig. 1) are isolated from plants and their characterization has been reported (Zhou et al., 1999; Ge et al., 2006, 2008, 2009, 2010; Qin et al., 2011). Antibodies against Erk2 (sc-154) and phospho-Erk1/2 (p-Erk1/2, sc-101760) were purchased from Santa Cruz Biotechnologies (San Cruz, CA). Antibody against IκBα (9242S) was purchased from Cell Signaling (Beverly, MA). Anti-GAPDH monoclonal antibody (MB001) was purchased from Bioworld Technology (Minneapolis, MN). Rabbit antiserum against HSV-2 ICP0 protein was prepared using a commercial source (Abmart, Shanghai). *N*-acetylcysteine (NAC) and Anti-GFP (AG279) antibody were purchased from Beyotime Institute of Biotechnology (Haimen, China). Recombinant human TNFα was purchased from Sino Biological Inc. (Beijing, China). HRP-conjugated secondary antibodies and chemical reagents including resveratrol, anisomycin, acyclovir (ACV) and MTT were purchased from Sigma–Aldrich (Shanghai). The SuperSignal ECL reagent kit was purchased from Pierce/Thermo Fisher (Thermo Fisher, Rockford, IL).

2.3. Cytotoxicity assay

The cytotoxic effect of compounds was assayed using a MTT assay by measuring cellular metabolic activity in Vero cells (Mosmann, 1983). Briefly, Vero cells in 96-well plates were treated with or without a compound at 30 μM in duplicate samples initially. After 72 h incubation, MTT was added to each well to a final concentration of 0.5 mg/ml and incubated for another 4 h. The formazan crystals were dissolved in DMSO after removal of culture

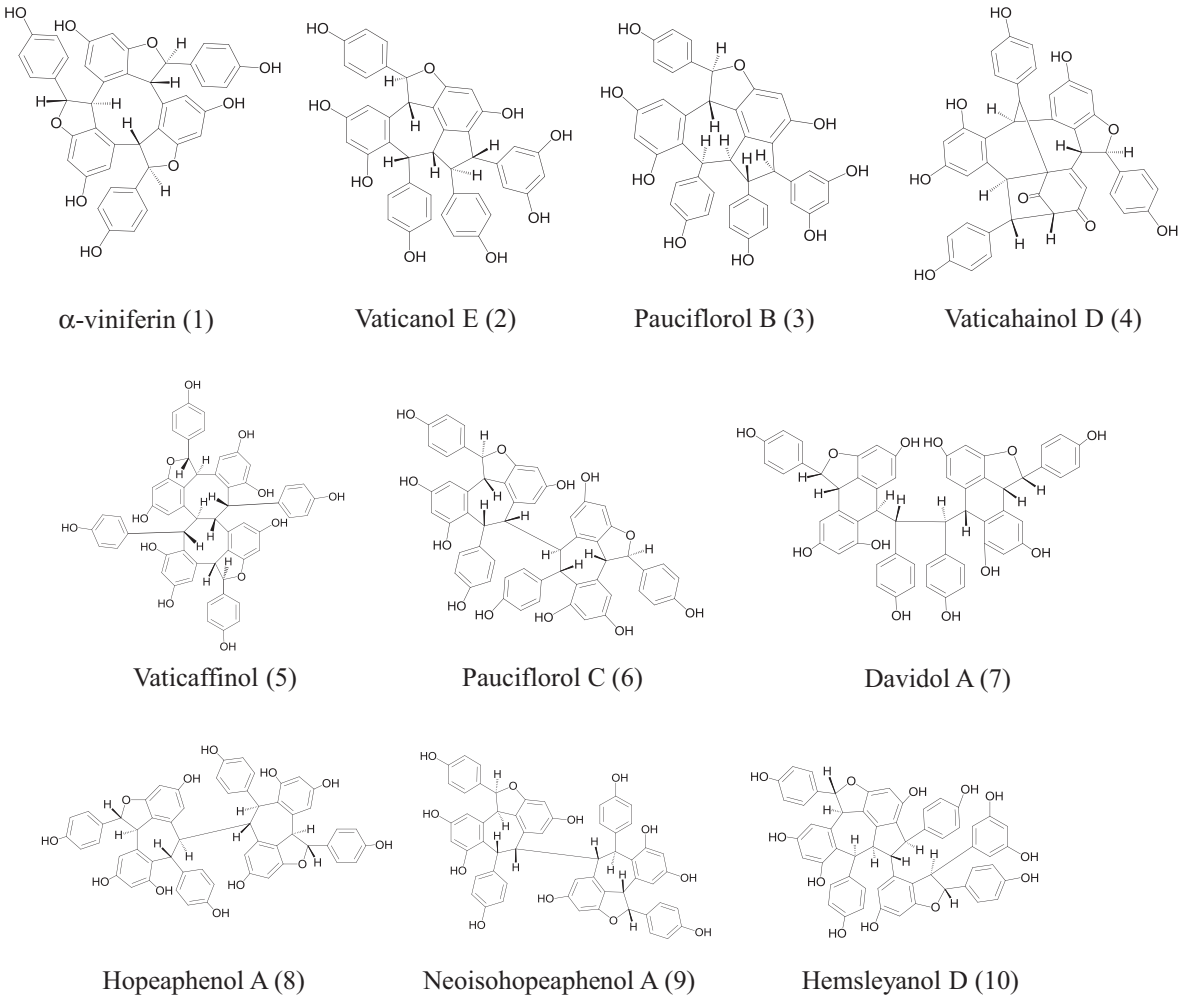

Fig. 1. Chemical structures of compounds used for anti-HSV studies.

medium and the absorbance was measured at 570 nm in a Versa Max microtiter plate reader (Molecular Devices, Sunnyvale, CA). Compounds that caused cytotoxic effect at 30 μM, were then tested at 3, 10 and 20 μM to obtain nontoxic concentrations in Vero cells.

2.4. Infection and inhibition assays

HSV infection was assayed initially by measuring cytolytic effect using MTT assay for convenience. Briefly, Vero cells in 96-well plates were treated with or without a test compound at 10 μM initially for 2 h in duplicate. The cells were then infected with HSV-1 or HSV-2 (MOI = 1 PFU/cell) without removal of the test compound. HSV infection caused cytolytic effect that was monitored under an inverted microscope and was quantified by measuring cell viability using the MTT assay at 72 h PI. Compounds with antiviral activities at 10 μM were subjected to secondary screening using concentrations of 3, 10 and 30 μM in duplicate samples to determine an IC_{50} using Graphpad Prism.

To verify antiviral effect determined by measuring cytolytic effect, plaque-forming assays were performed in some cases. Culture supernatants collected from HSV-infected samples were series-diluted and used to infect monolayers of Vero cells seeded in complete medium containing 2% fetal bovine serum. Plaque formation was assessed after 5 days by staining with crystal violet after fixation of the cells with 3% formaldehyde. The number of plaques was counted manually. The PFU numbers were presented as average of duplicate samples.

2.5. Western blot assay

HSV-infected cells or cells treated with 1 ng/ml TNFα or anisomycin (5 μM for 20 min) were harvested with a lysis buffer containing 150 mM NaCl, 50 mM Tris–HCl (pH 7.4), 1 mM sodium vanadate, 1% NP-40, and a cocktail of protease inhibitors (Roche). The lysates were centrifuged at 10,000*g* for 15 min to remove cellular debris. Soluble proteins were separated by SDS–PAGE under reducing conditions. After being transferred to a polyvinylidene difluoride membrane (PVDF, Millipore), the proteins were detected by incubation with a primary antibody, followed by horseradish peroxidase-conjugated secondary antibody and the SuperSignal ECL reagent kit. The images were collected using Alpha Innotech Flour Chem-FC2 imaging system (San Leandro, CA).

2.6. Flow cytometry assay

Vero cells were treated with or without a test compound prior to HSV-1/EGFP (MOI = 1 PFU/cell) infection. At the end of the experiment, cells were rinsed with 1× 0.25% trypsin–EDTA (Invitrogen). The cells were left at RT for 5 min to have cells detached with residual trypsin–EDTA. The cells were then collected, washed 2× with complete medium and then resuspended in PBS. GFP-positive cells were analyzed by flow cytometry analysis (FACSAria, BD Biosciences).

Intracellular ROS level was determined using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) according to a protocol provided by the manufacturer (Beyotime, Haimen, China). Briefly, the cells were pretreated with or without a test compound with indicated concentrations and times. The cells were then rinsed 3× with serum free medium and then fed with 5 μM DCFH-DA fluorescent probe in serum free medium for another hour. At the end of incubation, cells were washed 3× with PBS, and collected by trypsin–EDTA treatment. ROS production was measured by flow cytometry with excitation wavelength at 488 nm and emission wavelength at 525 nm and the results were analyzed by FCS Express v3 software.

3. Results

3.1. Inhibition of HSV infection by oligomeric stilbenoids

We undertook a project to evaluate antiviral effect of naturally occurring resveratrol derivatives using herpes simplex virus-1 and -2 as model system. The compounds (Fig. 1) were isolated from plants of *Hopea* genus and the identification of their identity has been described in previous publications (Ge et al., 2006, 2009; Qin et al., 2011). The cytotoxicity was evaluated on Vero cells using 30 μM or lower concentrations if a compound exhibited cytotoxic effect initially (Fig. 2A). For antiviral study, we measured cytopathic effect using a MTT assay (Fig. 2B). All compounds were tested with a non-toxic dose of 10 μM in Vero cells, more doses were tested to determine IC_{50} values for compounds with anti-herpetic activity in the initial assay. We also included resveratrol as a control since the compound was reported with anti-HSV-1 and HSV-2 activity (Faith et al., 2006; Docherty et al., 2004; Docherty, 2005). We used 30 μM since prolonged treatment of Vero cells with resveratrol at high concentrations tends to cause cell death. Resveratrol and several dimeric stilbenoids at up to 30 μM did not exhibit anti-HSV activity. Several trimeric and tetrameric derivatives showed activity against HSV-2 infection at micromolar concentrations (summarized in Table 1). Those compounds also showed antiviral activity against HSV-1 infection (Table 1). The compounds in general were more active against HSV-2 infection than that of HSV-1.

The antiviral activity was verified with immunoblotting analysis by detection of HSV-2 ICP0 expression or by FACS analysis by detection of GFP protein expression using a HSV-1 that encodes GFP reporter gene using vaticaffinol (compound 5), a compound that has been reported with antifungal activity. As shown in Fig. 2C and 2D, vaticaffinol treatment dose-dependently suppressed both HSV-1 and HSV-2 infection.

3.2. Oligomeric stilbenoids do not block NF-B or Erk/MAPK activation required for herpes simplex virus replication

The replication of HSV-1 and HSV-2 requires transcriptional activation and signaling transduction of the host cells (Patel et al., 1998; Gregory et al., 2004; Knipe and Cliffe, 2008; Zhang et al., 2010). Suppression of NF-κB transcriptional activity or MAPK signaling pathway has been demonstrated to inhibit HSV replication (Chen et al., 2011; Gregory et al., 2004; Amici et al., 2001). To elucidate a mechanism(s) on inhibition of HSV infection by oligomeric resveratrol derivatives, we first investigated whether those compounds blocked cellular signaling events. Both HSV-1 and HSV-2 infection promote NF-κB and MAPK activation. As shown in Fig. 3A, HSV-2 infection caused reduced detection of IκBα protein, indicating HSV infection promoted IκBα degradation, a prerequisite of NF-κB activation. Pretreatment with compounds 3 and 5 blocked IκBα degradation in HSV infected cells. We realize that an absence of NF-κB and MAPK activation in the treated samples does not necessarily translate into inhibition of NF-κB and MAPK pathways since the oligomeric stilbenoids blocked HSV replication. We therefore investigated whether the compounds blocked TNFα-induced IκBα degradation as well as anisomycin-induced MAPK activation. As shown in Fig. 3B and C, pretreatment of Vero cells with the stilbenoids did not block NF-κB and MAPK activation induced by TNFα and anisomycin, respectively.

3.3. Oligomeric stilbenoids inhibit herpes simplex virus infection independent of p53 protein expression

It has been reported that resveratrol promotes p53 accumulation and activation (She et al., 2001; Giaccia and Kastan, 1998).

The tumor suppressor p53 has been widely known as 'the guardian of the genome' due to its ability to prevent the occurrence of cancerous transformation of cells by induction of cell cycle arrest and apoptosis. Recent studies indicate that p53 is also a direct transcriptional target of type I interferons (IFNs) and thus, is activated upon viral infection (Munoz-Fontela et al., 2011; Rivas et al., 2010). To determine whether oligomeric stilbenoids inhibited HSV infection through p53 modulated signaling, we tested the anti-herpetic effect of those compounds in H1299 cells, a human non-small cell lung carcinoma. H1299 cell line is well documented to have a homozygous partial deletion of the *TP53* gene and as a result, does not express the p53 protein (Lin and Chang, 1996). Treatment with the oligomeric stilbenoids 3 and 5 effectively blocked HSV infection in H1299 cells (Fig. 4), indicating those compounds block HSV infection independent of p53 protein.

3.4. Oligomeric stilbenoids promote rapid but transient ROS generation

Recent studies by Gonzalez-Dosal et al. revealed that the host produces ROS in response to HSV-1 and HSV-2 infection (Gonzalez-Dosal et al., 2011, 2012). Polyphenolic compounds including resveratrol derivatives are known as effective antioxidants and some have been shown with potent antiviral activity (Fang, 2002; Wood et al., 2010). To preliminarily determine whether oligomeric stilbenoids accomplished antiviral activity through ROS pathway, Vero cells were treated with the stilbenoids at 10 μM. Intracellular ROS production was measured via 2′,7′-dichlorofluorescin diacetate (DCFH-DA) oxidation. Unexpectedly, we detected significant increase of ROS production in samples treated with compound 5 (Fig. 5A, upper panel) and oligomeric stilbenoids that showed antiviral activity (Fig. 5A, lower panel). ROS production was specific since inclusion of *N*-acetylcysteine (NAC), a commonly used scavenger of ROS (Zafarullah et al., 2003), reduced DCFH-DA oxidation (Fig. 5B).

ROS generation can lead to antimicrobial response or tissue injury, depending on the absolute intracellular levels of ROS and other reactive species or the temporal length of the alteration (Soberman, 2003; Grandvaux et al., 2007; Novo and Parola, 2008). In addition, we also performed studies to determine a time course of ROS generation using compound 5 as a model compound. As shown in Fig. 5C, ROS production was detected as early as 30 min post treatment. ROS production reached a peak value within 2 h following drug treatment, and rapidly returned to background level. At 6 h post treatment, ROS levels were detected

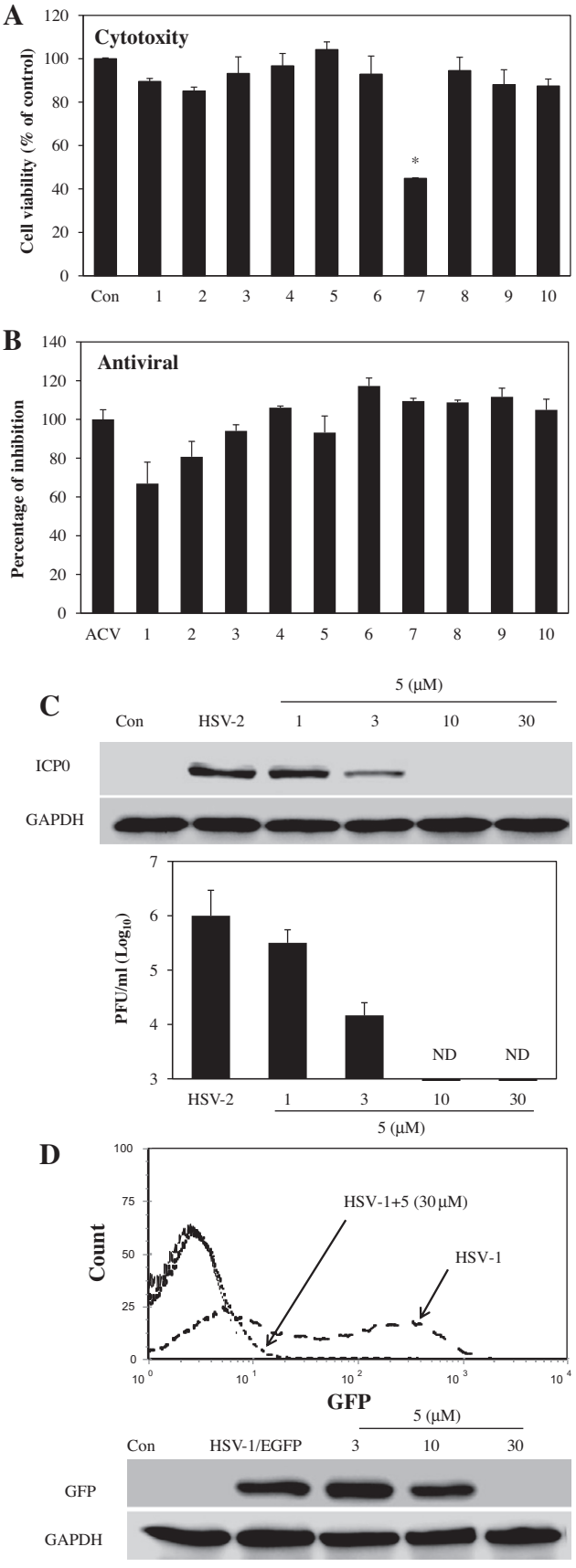

Fig. 2. Cytotoxicity assay and anti-herpetic activity assay of oligomeric stilbenoids. (A) The cytotoxicity was measured in Vero cells using an initial concentration of 30 μM in 96-well plates. Cell proliferation was measured by MTT assay after 72 h. * Indicates that compound 7 at 30 μM showed significant toxicity. The nontoxic dose was determined in Vero cells as 17.0 ± 3.0 μM or less from subsequent studies. (B) The anti-HSV-2 effect of oligomeric stilbenoids was measured as the inhibition of viral cytopathic effect using MTT assay using duplicate samples. Monolayers of Vero cells in 96-well plates were pretreated with or without 10 μM compounds for 2 h, and then infected with HSV-2 at an MOI of 1 PFU/cell. Cell viability was measured at 72 h PI. The data are presented as percentage inhibition of cytopathic effect by virus infection using the OD values of infected and untreated samples as no inhibition and uninfected and untreated controls as 100% inhibition. Acyclovir (ACV) at 10 μM showed a rate of 100% inhibition. (C and D) Stilbenoids dose-dependently blocked HSV-1 and HSV-2 infection. Vero cells were pretreated with compound 5 at concentrations as indicated and then infected with an HSV-1 harboring EGFP or HSV-2 (MOI = 1 PFU/cell). Cells were collected at 48 h PI for detection of HSV-2 ICP0 expression by Western blot analysis (C, upper panels) or for EGFP expression determined by FACS analysis and Western blot (D). GAPDH was used as loading control. In parallel experiments, the production of HSV-2 in compound 5-treated samples was verified using a secondary infection assay (C, lower panel). Pretreatment of Vero cells with compound 5 at 10 and 30 μM completely blocked progeny production of HSV-2, while at 1 and 3 μM HSV-2 production was reduced by 0.5 and 1.8 logs, respectively. Data are presented as mean ± standard error of duplicate samples. Results are representative of three independent experiments. Con: DMSO (0.1%) mock-treated control.

Table 1
Antiviral activity of stilbenoids against HSV-1 and HSV-2. The IC_{50} values were determined by measuring inhibition of cytopathic effect caused by HSV infection. Other compounds tested but showed no activity at 30 μM and not listed here include resveratrol, piceid (resveratrol-3-β-D-glucoside, polydatin), (+)-ε-viniferin (dimeric), hopeafuran (dimeric), pauciflorol E (dimeric), and hopeahainanphenol (dimeric).

Compound	IC_{50}	
	HSV-1 (μM)	HSV-2 (μM)
α-Viniferin (1, tri-)	27.4 ± 0.5	4.2 ± 0.4
Vaticanol E (2, tri-)	12.1 ± 0.5	4.5 ± 0.1
Pauciflorol B (3, tri-)	16.7 ± 0.5	3.2 ± 0.5
Vaticahainol D (4, tri-)	12.2 ± 0.5	4.5 ± 0.2
Vaticaffinol (5, tetra-)	17.9 ± 0.3	3.2 ± 0.3
Pauciflorol C (6, tetra-)	24.1 ± 0.6	3.3 ± 0.1
Davidol A (7, tetra-)	27.9 ± 0.6	3.3 ± 0.2
Hopeaphenol A (8, tetra-)	17.3 ± 0.5	3.7 ± 0.2
Neoisohopeaphenol A (9, tetra-)	8.7 ± 0.4	3.4 ± 0.3
Hemsleyanol D (10, tetra-)	9.1 ± 0.5	3.8 ± 0.2

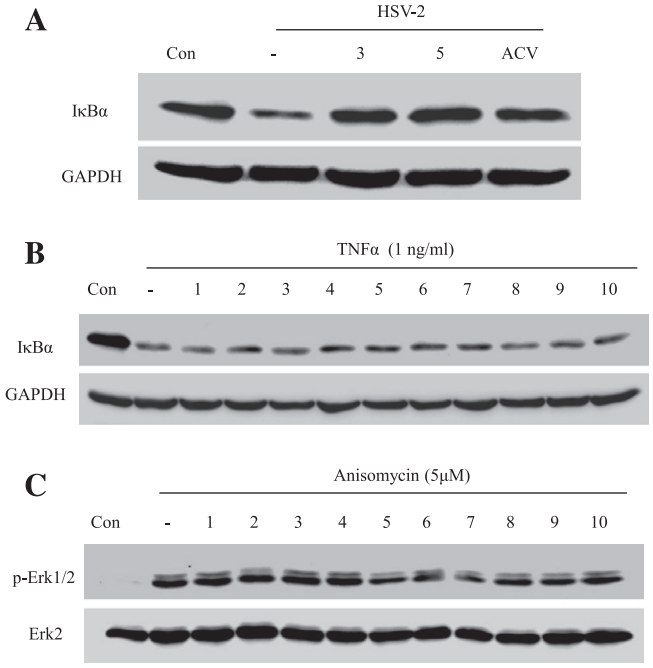

Fig. 3. No inhibitory effect of oligomeric stilbenoids on NF-κB and Erk/MAPK activation. Vero cells infected with HSV-2 (MOI = 1 PFU/cell) in the presence or absence of 10 μM compounds 3 and 5. Cell lysates were harvested at 24 h PI, degradation of inhibitory protein IκBα was determined by Western blot analysis as a measure of NF-κB activation. Acyclovir (ACV) at 10 μM was used as positive control (A). In separate experiments, the effect of oligomeric stilbenoids on TNFα-induced (1 ng/ml) IκBα degradation (B) or mitogen-induced Erk phosphorylation (anisomycin at 5 μM for 20 min) was tested on HeLa cells at 10 μM (C). GAPDH expression was used as a loading control. Con: DMSO mock-treated control. Results are representative of three independent experiments.

close to untreated samples. These data indicated that oligomeric stilbenoids promoted rapid but transient ROS response.

3.5. Oligomeric stilbenoids inhibit HSV infection through ROS generation

ROS play a critical role in innate immunity and antiviral response. We therefore investigated whether oligomeric stilbenoids inhibited HSV infection through induction of ROS. To this end, Vero cells were first treated with NAC to quench ROS produced by stilbenoid treatment. The cells were then infected with HSV-1 or HSV-2 for 48 h and virus infection was determined by immunoblotting analysis or by FACS. NAC alone showed no effect on HSV

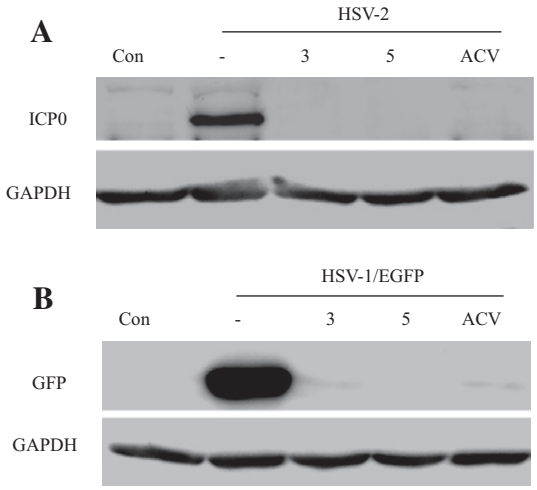

Fig. 4. Independency of p53 status of stilbenoid anti-herpetic activity. The p53-null H1299 cells were pretreated with compounds 3 or 5 for 2 h and then infected with HSV-2 (A, at 10 μM) or HSV-1/EGFP (B, at 30 μM) at MOI = 1 PFU/cell. Cells were collected at 48 h PI for detection of HSV-2 ICP0 or EGFP expression by Western blot analysis. Acyclovir (ACV) at 10 μM was used as a positive control. Results are representative of three independent experiments. Con: DMSO mock-treated control.

replication. Addition of NAC dose-dependently reversed the inhibitory effect of compound 5 on HSV infection (Fig. 6), demonstrating oligomeric stilbenoids inhibited HSV replication through ROS generation.

4. Discussion

Resveratrol is a polyphenolic natural product that is present in red wine and peanuts and has inhibitory activity against inflammation, heart disease, and cancer. Similar to resveratrol, oligomeric stilbenoids are polyphenolic phytoalexins produced by plants in response to biotic or abiotic stress. Stilbenoids, particularly resveratrol, have received much attention because of their significant biological effects. Here we tested the antiviral effect of anti-herpetic activity of oligomeric resveratrols. Our results show a potent, dose-dependent antiviral effect of resveratrol derivatives in vitro. The antiviral effect of those compounds was demonstrated at significantly reduced concentrations that do not induce cytotoxicity in cultured cells. Moreover, these compounds achieved significant reduction in viral titers and the immediate early gene expression.

The demonstrated benefits of resveratrol may be associated with its general activity as a modulator of the transcription factor NF-κB, induction of apoptosis, and possibly activation of SIRT1. The replication of HSV-1 and HSV-2 requires activation of host signal transduction machinery, including MAPK and NF-κB activation and histone remodeling. Genetic manipulation leading to blockage of MAPK activation has been shown to suppress HSV-2 infection (Zhang et al., 2010). Chemical reagents that inhibit NF-κB activation block HSV-1 and HSV-2 infection. The oligomeric stilbenoids showed no activity against NF-κB activation induced by HSV-2 infection or by TNFα stimulation or MAPK activation by anisomycin. Instead, we found that the stilbenoids promoted ROS production. Inclusion of NAC, a ROS scavenger molecule, reversed the inhibitory effect of stilbenoids against HSV infection, demonstrating those compounds suppressed HSV infection through ROS generation.

Oxidative damage is a common feature associated with virus infection (Valyi-Nagy and Dermody, 2005; Spooner and Yilmaz, 2011). As important players of innate immunity, ROS also are beneficial for the host to limit viral replication. Gonzalez-Dosal

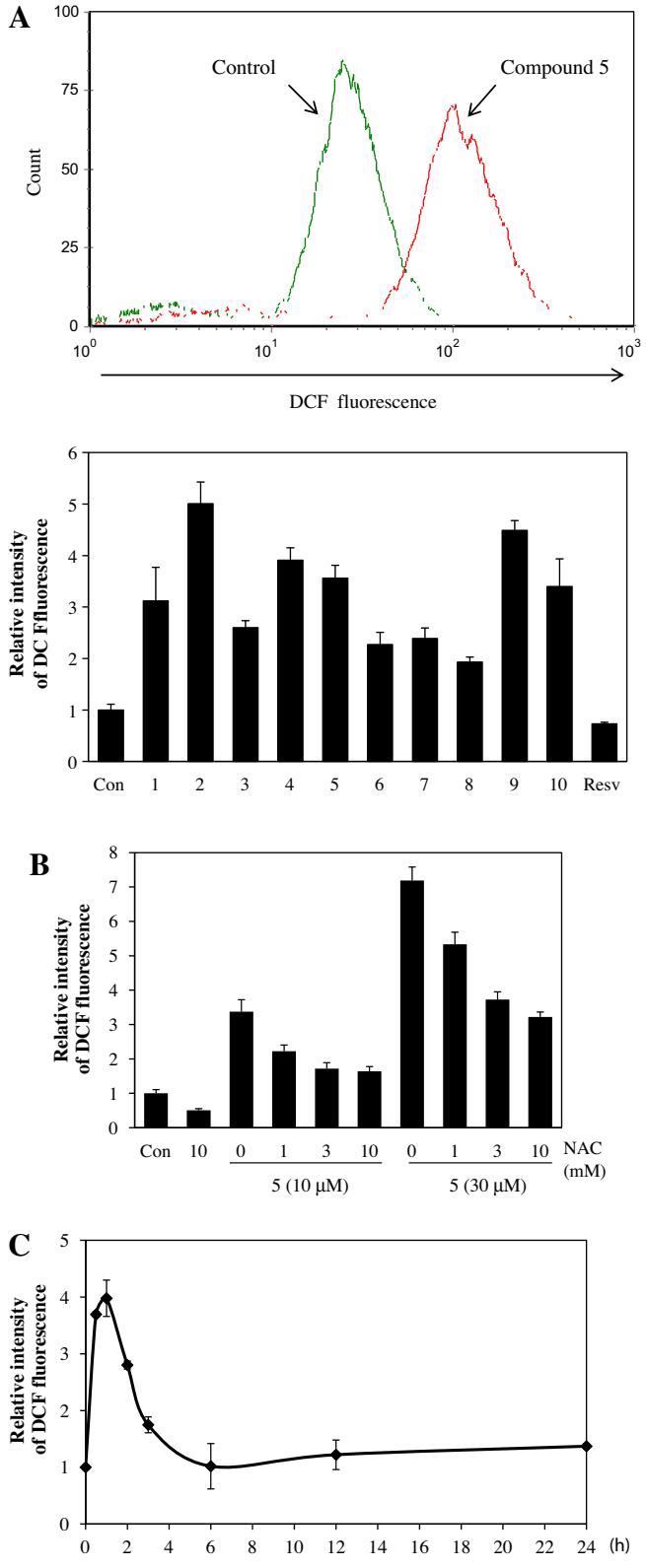

Fig. 5. Oligomeric stilbenoids promote ROS generation. Intracellular ROS production was measured by FACS analysis after incubation with DCFH-DA. Vero cells were treated with or without compounds (10 μM) for 1 h and then with 5 μM DCFH-DA for another hour. DCF fluorescent intensity was quantified by FACS (A). Vero cells treated with 1 mM, 3 mM, or 10 mM *N*-acetylcysteine (NAC) for 1 h prior to stimulation with compound 5 at 10 μM or 30 μM. Intracellular ROS levels were detected by FACS after incubation for another hour (B). Time course of ROS generation after treatment with compound 5 (C). The data are presented as mean ± standard error of duplicate samples, and the results are representative of three independent experiments. A duplicate sample of cells fed with 5 μM DCFH-DA was used as a control (Con).

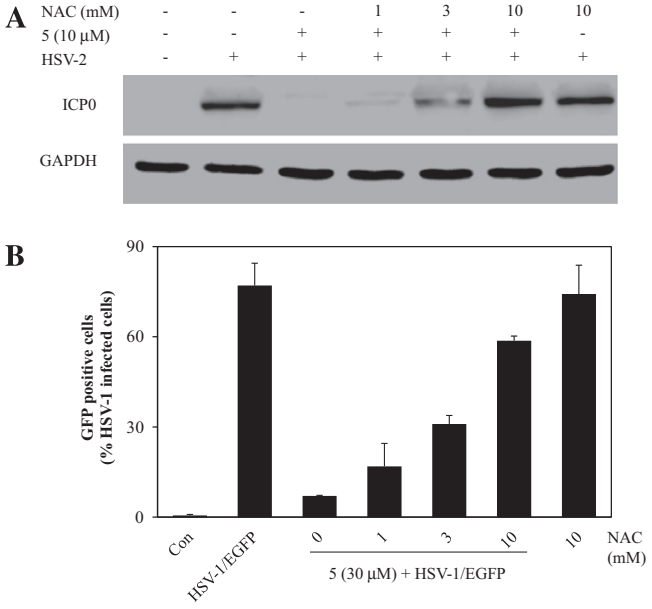

Fig. 6. *N*-acetylcysteine reverses the inhibitory effect of stilbenoids on HSV replication Prior to HSV-1 or HSV-2 infection, Vero cells were treated with 1 mM, 3 mM, 10 mM NAC for 1 h, followed by compound at 10 μM or 30 μM for another hour. The cells were harvested at 48 h PI and subjected to Western blot analysis for HSV-2 ICP0 expression (A) or for the detection of GFP-positive cells by FACS (B). NAC at 10 mM showed no effect on HSV-1 and -2 infection. The data are presented as mean ± standard error of duplicate samples, and the results are representative of three independent experiments. DMSO (0.1%)-treated samples were used as controls (Con).

and colleagues have recently shown that HSV-1 infection induces oxidative stress and the release of bioactive lipid peroxidation by-products in mouse neural cell cultures (Kavouras et al., 2007; Gonzalez-Dosal et al., 2011). The production of ROS potentiates signaling from pattern recognition receptors for ROS-dependent activation of innate immune pathways (Gonzalez-Dosal et al., 2011). We found that oligomeric stilbenoids treatment promoted ROS production, coinciding with suppression of HSV-1 and HSV-2 replication in those samples.

It is intriguing to note that induction of ROS has been recognized as a common mechanism against virus infection. We were surprised that those compounds showed no activity against enterovirus 71 (Data not shown), an RNA virus that causes hand foot and mouth disease in infants and children. Gonzalez-Dosal reported recently that mitochondria-derived reactive oxygen species negatively regulates innate immune signaling pathways triggered by HSV but not by an RNA virus (Gonzalez-Dosal et al., 2012). The host utilizes TLR2 and TLR9 to sense HSV infection (Lund et al., 2003; Sato et al., 2006; Rasmussen et al., 2009; Martinez-Martin and Viejo-Borbolla, 2010). Whether those compounds target TLR signaling remains to be determined. Nonetheless, the identification of oligomeric resveratrol derivatives with anti-herpetic activity expands our knowledge of biological activities of naturally occurring stilbenoids.

Acknowledgments

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