Resveratrol inhibition of herpes simplex virus replication

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Abstract

Resveratrol, a phytoalexin, was found to inhibit herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) replication in a dose-dependent, reversible manner. The observed reduction in virus yield was not caused by the direct inactivation of HSV by resveratrol nor inhibition of virus attachment to the cell. The chemical did, however, target an early event in the virus replication cycle since it was most effective when added within 1 h of cell infection, less effective if addition was delayed until 6 h post-infection and not effective if added 9 h post-infection. Resveratrol was also found to delay the cell cycle at $S-G_2-M$ interphase, inhibit reactivation of virus from latently-infected neurons and reduce the amount of ICP-4, a major immediate early viral regulatory protein, that is produced when compared to controls. These results suggest that a critical early event in the viral replication cycle, that has a compensatory cellular counterpart, is being adversely affected. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Herpes simplex virus (HSV) is a common human virus that afflicts the majority of the population (Whitley and Gnann, 1993). Infection with HSV normally results in limited benign lesions; but, in some instances, it is capable of causing serious, life-threatening disease or blindness (Spruance et al., 1977; Whitley et al., 1981; Corey et al., 1983; Cobo, 1988). A normal sequela to a primary infection is the establishment of latency as the virus takes up permanent residence in the ganglia of the host (Stevens and Cook, 1971). This location then can serve as a site for reoccurring infections which, in turn, may become a source of infection for an unwitting host.

HSV is a DNA virus, and successful chemotherapeutic intervention has commonly targeted the DNA synthesizing machinery of this virus. Indeed, the agent most commonly used to treat HSV, acyclovir, targets viral DNA polymerase through virus-specific thymidine kinase to effectively disrupt viral DNA synthesis and ultimately the replication of HSV (Elion, 1996). Most

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other drugs which have been used to target HSV infections are also nucleoside analogues, but have properties that make them less effective or more toxic than acyclovir and, therefore, less desirable for use in treatment.

An ongoing concern in anti-HSV chemotherapy is the development of viral resistance. Indeed, strains of HSV resistant to acyclovir and nucleoside analogues have been reported (Coen and Schaffer, 1980; Crumpacker et al., 1980, 1982; Oliver et al., 1989). It is therefore of some considerable importance that new and novel chemotherapeutics to treat HSV infections continually be developed.

In this regard, we report here that resveratrol (3,5,4′-trihydroxystilbene), a naturally-occurring anti-fungal phytoalexin found in grapes and other plants (Hain et al., 1990; Schubert et al., 1997; Soleas et al., 1997), effectively inhibits HSV-1 and HSV-2 replication. This compound is present in red wines and is reported to have a variety of physiological effects. Its antioxidant activity is thought to protect against coronary artery disease through the modulation of lipid synthesis (Frankel et al., 1993) and inhibition of platelet aggregation (Pace-Asciak et al., 1996). In addition, a recent report described it as a cancer chemo-preventative, affecting the three major stages of carcinogenesis (Jang et al., 1997).

Our studies demonstrate that, when added to culture media, resveratrol adversely affects HSV replication when present during the first 6 h of infection, reversibly inhibiting an early event in the viral growth cycle. This reduction in progeny virus is not caused by direct inactivation of the virus by resveratrol nor inhibition of virus attachment to the cell. We also show that resveratrol inhibits reactivation of latent HSV from explanted trigeminal ganglia and that it arrests cells in the S–G_2–M phase of growth.

2. Materials and methods

2.1. Cells and virus

African green monkey kidney cells (Vero) and human diploid lung cells (MRC-5) were obtained from the American Type Culture Collection, Rockville, MD. Vero cells were grown and maintained in Medium 199 supplemented with 5% fetal bovine serum, 0.075% NaHCO$_3$, and 50 μg/ml gentamycin sulfate. MRC-5 cells were grown and maintained in Eagle's basal medium supplemented with 10% fetal bovine serum, 0.075% NaHCO$_3$, and 50 μg/ml gentamycin sulfate.

HSV-1 and HSV-2 were isolated from two different patients and were previously characterized (Zimmerman et al., 1985). Vero cells were used to produce virus pools and also in plaque assays (Docherty et al., 1971). Viral replication studies in the presence or absence of drugs were performed in Vero and MRC-5 cells.

2.2. Chemicals

Resveratrol (3,5,4′-trihydroxystilbene) and piceatanol (3,3′,4,5′-tetrahydroxystilbene) were purchased from Sigma Chemical, St. Louis, MO.

Stock concentrations of resveratrol and piceatanol were prepared in 100% ethanol and diluted to final concentrations in tissue culture media. The highest final concentration of alcohol in tissue culture media was 1% and occurred when resveratrol was used at a concentration of 100 μg/ml. Separate HSV replication studies in the presence of 0.5% ethanol demonstrated that it had no effect on virus replication and yield, whereas 1% ethanol slightly reduced viral yields.

2.3. Viral replication studies

Cells in culture were infected with HSV-1 or HSV-2 at a multiplicity of infection (moi) of one. After 1 h of adsorption, the cells were rinsed once with media and then media, with or without the test chemical, was added at the stated concentration. At that time, 0 h samples were frozen at −70°C and every 24 h thereafter up to 72 h. At the end of that period, samples containing supernatant and cells were thawed, sonicated, and titrated on Vero cells.

2.4. Toxicity study

Vero cells were exposed to 50 or 100 µg/ml of resveratrol for varying periods of time up to 24 h. At various time points, trypan blue dye exclusion cell counts were performed according to standard methods (Strober, 1997) to determine if cells were adversely affected by resveratrol.

2.5. Immediate–early viral protein, ICP-4 synthesis

Vero cells were infected with HSV-1 for 24 h. At that time, cells were scraped from the flask, collected by centrifugation, and resuspended in cold tris buffered saline (TBS). The cells were pelleted by centrifugation, TBS removed, and the cell pellet frozen at $-70°C$. The pellets were thawed, resuspended in cold RIPA buffer (Gilman et al., 1980) and sonicated one minute. DNase (50 µg/ml) was added to the suspension which was then incubated on ice for 30 min prior to clarification by centrifugation at $16\,000 \times g$.

Proteins from the infected cell extract were separated by 6–15% SDS-PAGE and electrophoretically transferred to nitrocellulose. The ICP-4 protein was immunochemically detected by reacting the blotted proteins with mouse monoclonal antibody to ICP-4 (Goodwin Institute for Cancer Research, FL), then peroxidase conjugated sheep anti-mouse antibody, and finally with diaminobenzidine and urea hydrogen peroxide (Sigma Fast, Sigma). A single high molecular weight band was observed at 169 kDa.

2.6. Latency studies

Thirty-two FVB (Taketo et al., 1991) mice were anesthetized with metofane and both corneas lightly scarified with a 30 gauge needle. A 50 µl inoculum of 10^4 pfu of HSV-1 was placed on the eye surface which was then closed and gently massaged. An inoculum of 10^4 pfu was used because preliminary studies using higher quantities of virus (i.e. 10^5–10^6 pfu) proved lethal. Using lower inoculum results in a higher survival rate, but lower reactivation rate, which is consistent with the studies of others (Sawtell, 1998). The animals were rested for not less than 30 days. The animals were euthanized and the left and right trigeminal ganglia were aseptically removed and placed in separate wells of a 12 well tissue culture plate. One-half of the ganglia were incubated in tissue culture media containing 50 µg/ml of resveratrol; the other half, with media alone. Each day for 10 days, the fluid from each well was drawn off, frozen, and replaced with fresh media. At the end of 10 days, each sample was tested for the presence or absence of infectious virus on Vero cells by the plaque assay.

2.7. Flow cytometry

To determine if resveratrol was affecting the cell cycle, Vero cells were incubated in media containing 50 µg/ml of resveratrol or media alone for 30 h. All cells were then trypsinized, reacted with propidium iodide and analyzed with a Cytoron Absolute Flow Cytometer (Vindelov et al., 1983).

3. Results

3.1. Resveratrol inhibition of herpes simplex virus replication

To determine the effect of resveratrol on HSV replication, Vero cells were infected with HSV-1 for 1 h and media containing 10, 25, or 50 µg resveratrol/ml was added to each flask. Infected control samples received media only. Samples taken every 24 h were titrated by the plaque assay and compared to the sample containing no drug. The results of one representative study are presented in Fig. 1a and demonstrate that, at 50 µg/ml, HSV-1 replication was severely impeded. At 24, 48 and 72 h, samples that were treated with 50 µg/ml of resveratrol had $>99\%$ less virus produced than controls. By 72 h, the virus was virtually undetectable in cultures continuously incubated in the presence of 50 µg/ml of resveratrol. Treatment with 25 µg/ml resveratrol reduced new virus production by as much as 95%, while treatment with 10 µg/ml of resveratrol had little-to-no effect on virus production when compared to

controls (data not shown). The studies were repeated twice more with essentially the same results. When the effect of resveratrol on HSV-2 replication was examined, the same inhibitory pattern was observed (Fig. 1b); that is, resveratrol at 50 µg/ml severely impeded HSV-2 replication. Similar to HSV-1, 10 and 25 µg/ml had limited effect on HSV-2 replication (data not shown).

To determine if cell type affected the ability of resveratrol to inhibit HSV-1 replication, these studies were repeated using MRC-5 cells. The results presented in Fig. 1c demonstrate that at 50 µg/ml of resveratrol, the inhibitory pattern of HSV-1 replication in MRC-5 cells is very similar to that seen in Vero cells.

These studies were repeated with HSV-1 using piceatanol, a stilbene that differs from resveratrol

Fig. 2. Resveratrol cell toxicity studies. To determine if resveratrol was toxic to cells, Vero cells were incubated with 50 µg/ml of the chemical from 1–24 h at 37°C. At each indicated time point, trypan blue dye exclusion studies were performed as an index of cytotoxicity. Results are expressed as percent viable cells.
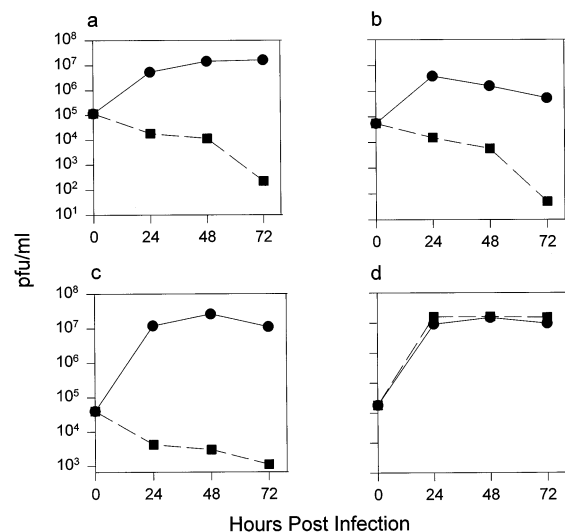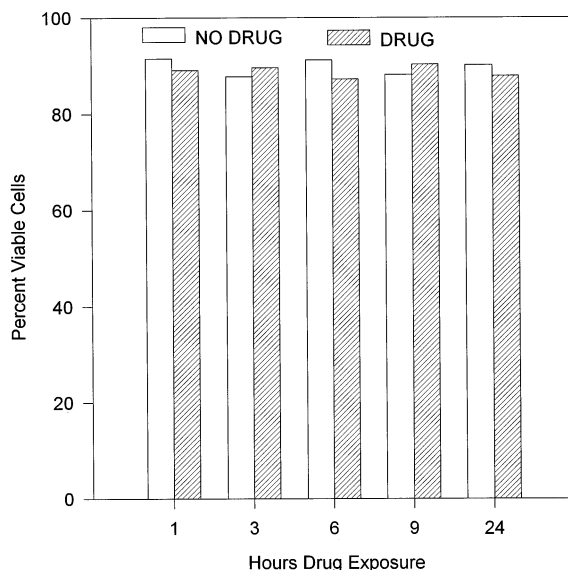

Fig. 1. HSV-1 or HSV-2 replication in the presence of resveratrol or piceatanol. Vero cells or MRC-5 cells were infected with HSV-1 or HSV-2 and incubated in the presence or absence of 50 µg/ml of resveratrol or piceatanol. At 24 h intervals, samples were taken and titrated by the plaque assay. Results are expressed as pfu/ml. Vero cells infected with HSV-1(a) or HSV-2(b) in the presence or absence of 50 µg/ml of resveratrol (c) MRC-5 cells infected with HSV-1 in the presence or absence of 50 µg/ml of resveratrol (d) Vero cells infected with HSV-1 in the presence or absence of 50 µg/ml of piceatanol. ●–● Control, ■–■ 50 µg/ml of resveratrol or piceatanol.

by one hydroxyl group. There was no effect on HSV-1 replication at 50 µg /ml piceatanol (Fig. 1d). These studies were repeated using piceatanol at a concentration of 100 µg/ml and, again, there was no effect on HSV-1 replication (data not shown).

3.2. Resveratrol cell toxicity studies

Trypan blue dye exclusion studies were used to determine if resveratrol was toxic to Vero cells and cell death could account for the reduced amount of virus produced. Results presented in Fig. 2 reveal that exposure to 50 µg/ml for 24 h of resveratrol was not toxic to Vero cells. These studies were repeated using 100 µg/ml and it was noted that there was an 18% reduction in viable cells after 24 h of exposure to this concentration of resveratrol (data not shown). Therefore, all further studies were done at a resveratrol concentration of 50 µg/ml.

3.3. Resveratrol does not directly inactivate herpes simplex virus

To determine if resveratrol was capable of directly inactivating HSV resulting in reduced virus yields, a standard inoculum of HSV was mixed with 50 μg/ml of resveratrol containing media, 0.5% ethanol containing media, or media only and placed at room temperature. Residual virus was titrated at 1, 30 and 60 min after mixing with the above formulations. As can be seen in Fig. 3, resveratrol had no direct inactivating effect on HSV during the time period examined.

3.4. Resveratrol does not block herpes simplex virus attachment

To determine if attachment of HSV to Vero cells was affected by resveratrol resulting in reduced viral yields, Vero cells were incubated with 50 μg/ml of resveratrol for 10 min. The chemical was then removed and the cells immediately ex-

Fig. 4. The effect of resveratrol on virus attachment. Vero cells were incubated with 50 μg/ml of resveratrol for 10 min or control media containing 0.5% EtOH. The drug was removed and the cells immediately exposed to HSV-1. Samples were taken at 24 h intervals and titered by the plaque assay. Results are expressed as pfu/ml.

posed to HSV-1 and control media lacking resveratrol. Samples were taken every 24 h for 72 h. The results in Fig. 4 show that HSV-1 replication was not affected by pre-exposure of cells to resveratrol. This study was repeated, but resveratrol was placed on the cells 10 min before the virus and kept on during the 1 h attachment period. At that time, unattached virus and drug were rinsed out and replaced with control media. Samples were taken every 24 h for 72 h and the results were the same as those in Fig. 4 (data not shown). Those results suggest that attachment is likely not the site at which resveratrol blocks HSV replication.

3.5. Early exposure to resveratrol is required for inhibition of herpes simplex virus replication

To approximate the time period in the replication scheme that resveratrol was effective, the chemical was added at various times after Vero cells were infected. The data in Fig. 5a reveals that resveratrol was most effective the earlier it was added to the cells after infection with HSV-1.
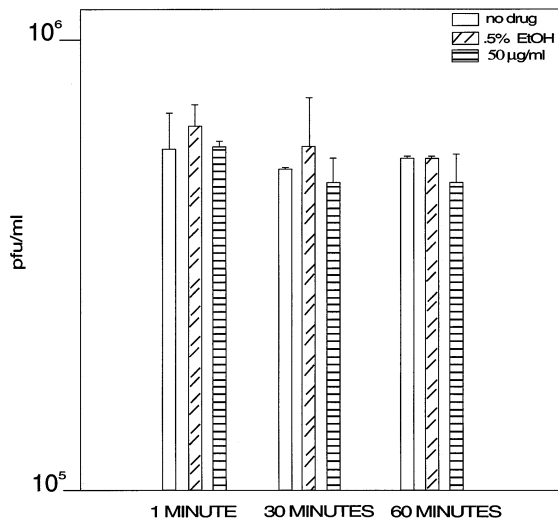

Fig. 3. Lack of direct inactivating effects of resveratrol on HSV-1. To determine if resveratrol is capable of directly inactivating HSV-1, the virus was incubated with 50 μg/ml of the chemical for 1, 30, and 60 min at room temperature. At the indicated intervals, an aliquot was removed and virus titrated by the plaque assay. Because the highest concentration of EtOH in the resveratrol preparation is 0.5%, a control containing only 0.5% EtOH and virus, but no resveratrol, was included. Results are expressed as pfu/ml ($\pm$) S.E.
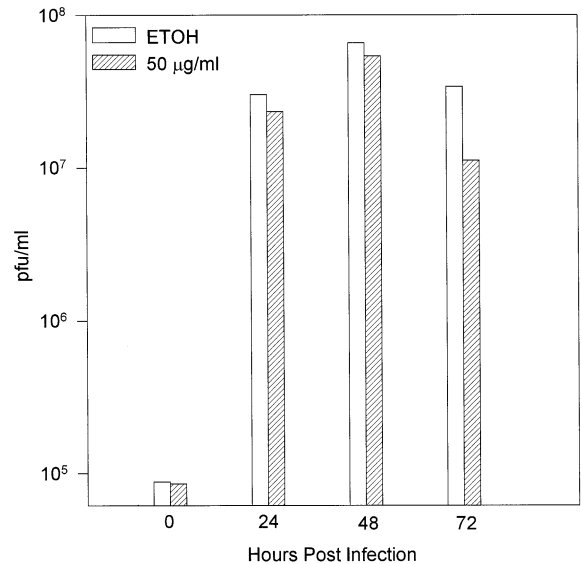
If it was added at 1 h after infection, > 99.9% of virus production was inhibited. If it was added 3 or 6 h after infection, virus replication was inhibited by ≈ 90%. If the drug was added 9 h after infection, there was no inhibitory effect of resveratrol on viral replication. When these studies were repeated with HSV-2, essentially the same results were obtained (Fig. 5b).

3.6. Inhibitory effects of resveratrol on herpes simplex virus type 1 replication are reversible

To determine if the observed inhibition of HSV replication was permanent or reversible, cells infected with HSV-1 were exposed to 50 μg/ml of resveratrol for a period of 72 h. One set of infected cells, however, had resveratrol containing media replaced with non-resveratrol containing media at 24 h, while another set had resveratrol containing media replaced with non-resveratrol containing media at 48 h. From the data presented in Fig. 6, it can be seen that as long as resveratrol was present, HSV-1 replication was severely depressed. However, when resveratrol was removed at 24 and 48 h, the virus proceeded to replicate in what appeared to be a normal fashion approaching, or reaching, levels of viral titers comparable to those of untreated cultures. The same results were obtained with HSV-2 (data not shown).

Fig. 6. Inhibitory effects of resveratrol on HSV-1 replication are reversible. Vero cells were infected with HSV-1 and incubated in control media or media containing 50 μg/ml of resveratrol. At 24 and 48 h, resveratrol containing media was replaced with non-resveratrol containing media. Samples were taken every 24 h and titered by the plaque assay. Results are expressed as pfu/ml.
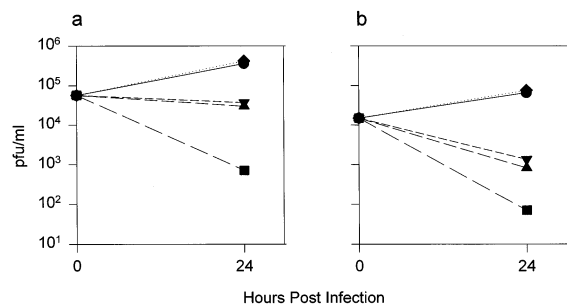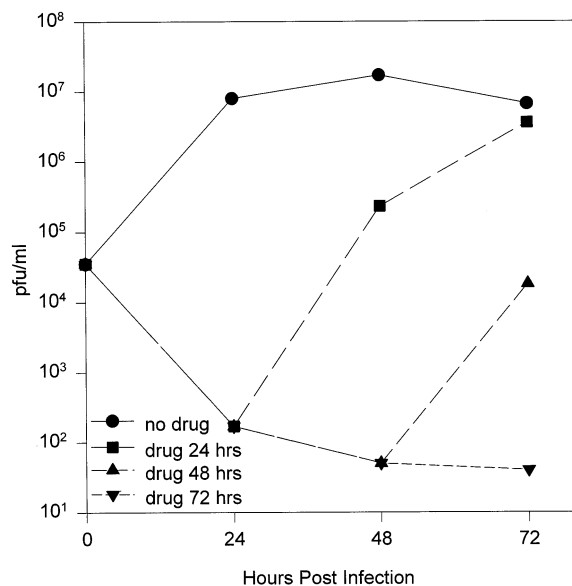

Fig. 5. Early Exposure of Infected Cells to Resveratrol is Required for Inhibition of HSV-1 and HSV-2 Replication. Vero cells infected with HSV-1(a) or HSV-2(b) were incubated with media containing 50 μg/ml of resveratrol beginning at 1, 3, 6, or 9 h after infection. All samples were frozen at 24 h and titrated by the plaque assay. Results are expressed as pfu/ml. ■–■, 1 h; ▲–▲, 3 h; ▼–▼, 6 h; ♦–♦ 9 h; ●–●, control.

3.7. Resveratrol limits ICP-4 production

Because our results suggested that an early event in the replication cycle of the virus was being affected, we tested for ICP-4, an immediate-early protein of HSV that is a major essential regulatory protein (DeLuca and Schaffer, 1985). Inhibition of ICP-4 can severely impede HSV replication (Dixon and Schaffer, 1980). Western immunoblots were utilized to examine infected cell extracts for the presence of ICP-4 in the presence and absence of resveratrol. It can be seen in Fig. 7 that when cells were examined for ICP-4 24 h after infection with HSV-1, the amount detected in resveratrol-treated cultures was considerably less than in cultures devoid of resveratrol.
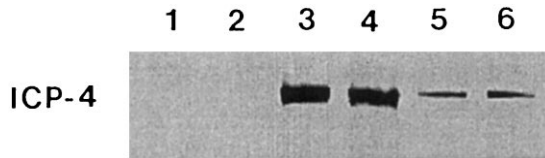
Fig. 7. The effect of resveratrol on ICP-4 formation. Vero cells infected with HSV-1 and incubated in the presence or absence of resveratrol for 24 h were solubilized, proteins separated on 6–15% SDS-PAGE, transferred to nitrocellulose and immunostained with monoclonal antibodies to ICP-4. Lanes 1 and 2—uninfected Vero cells. Lanes 3 and 4—HSV-1-infected Vero cells—resveratrol absent. Lanes 5 and 6—HSV-1-infected Vero cells—resveratrol present.

3.8. Resveratrol prevents reactivation of latent virus

ICP-4 is required for HSV replication in trigeminal ganglia (Bates and DeLuca, 1998). Since our previous study demonstrated that resveratrol reduces ICP-4 production, we examined latently-infected trigeminal ganglia for viral reactivation in the presence or absence of resveratrol. Both latently-infected trigeminal ganglia were excised from 32 mice and half were incubated in tissue culture media with 50 µg/ml of resveratrol and half in control media. Samples were removed daily for 10 days and tested for reactivated infectious virus by the plaque assay. It was found that HSV reactivated from 7 (22%) of the ganglia incubated in control media while no virus was recovered from ganglia incubated in resveratrol containing media ($P = 0.01$, Fisher Exact Test, 2-Tail, Table 1).

Table 1
The effect of resveratrol on reactivation of HSV from latently-infected trigeminal ganglia[a]

Treatment	Number Ganglia	Number ganglia reactivated
Media only	32	7
Media plus resveratrol	32	0

[a] Both trigeminal ganglia were removed from each mouse whose eyes were previously infected with HSV and incubated for 10 days in media with or without resveratrol. Samples were taken and tested daily for infectious virus. A positive reactivation is the appearance of infectious virus in any of the 10-day test samples ($P = 0.01$).

Table 2
The effects of resveratrol on the cell cycle

Treatment	%G_0–G_1	%S	%G_2–M
Control (no resveratrol)	88.6	7.1	4.4
Resveratrol, 30 h	74.7	16.4	9.0

3.9. Resveratrol arrests the cell cycle

To determine if resveratrol was also affecting the cell, cell cycle studies using flow cytometry were carried out. When cells were treated with resveratrol for 30 h, the number of cells in the S–G_2–M phase of growth was greater than twice that found in cultures that were not exposed to resveratrol, suggesting that resveratrol was also affecting the cell (Table 2).

4. Discussion

Studies presented here demonstrate that resveratrol, a phytoalexin found in various plants, but most notably grapes, is an inhibitor of HSV-1 and HSV-2 replication. The observed reduction in virus yield does not appear to be caused by the direct inactivation of HSV by resveratrol nor does it appear that the chemical prevents the virus from attaching to the cell. It does however seem to target an early event in the replication cycle since the chemical is most effective when added within 1 h of cell infection, less effective if addition is delayed until 6 h post-infection and not effective if added 9 h post-infection. In addition the immediate early regulatory protein ICP-4 is produced at levels considerably less than that which is produced when resveratrol is absent, reactivation from latently infected ganglia is inhibited and the chemical appears to retard the cells in the S–G_2–M phase of growth.

Resveratrol has been shown to have a large number of regulatory biological functions. It is reported to protect against atherosclerosis

through its antioxidant properties (Fauconneau et al., 1997), inhibit platelet aggregation (Pace-Asciak et al., 1995), and down-regulate prostaglandin and prostacyclin synthesis probably due to the inhibition of cyclooxygenase and hydroperoxidase activity (Jang et al., 1997). It has also been shown to inhibit cellular events associated with tumor initiation, promotion, and progression as well as ribonucleotide reductase activity (Fontecave et al., 1998) and DNA polymerase activity (Sun et al., 1998). However, even though it has been extensively studied, the molecular mechanism of resveratrol's action is not clear and is likely variable depending on the system under investigation.

The replication of HSV is equally complex, requiring both cellular and viral factors coordinately regulated and sequentially activated to successfully produce fully infectious progeny. In a newly-infected cell, virion protein 16 (VP16) interacts with cellular factors Oct-1 and HCF to activate transcription of immediate early genes (O'Hare, 1993). In addition, recent studies have demonstrated that two inhibitors of cyclin-dependent kinase 1 (cdk-1) and cdk-2 negatively affect the accumulation of immediate early and early HSV transcripts depressing HSV replication. These studies suggest that a cellular function expressed in the late G_1/S phase of the cell cycle is required for HSV replication (Schang, et al., 1998).

Considering the wide range of effects ascribed to resveratrol and the complexity of HSV replication involving both viral and cellular factors, the possible target(s) that would account for the observed inhibition of HSV replication by resveratrol are considerable. Initially, we considered the antioxidant properties of resveratrol as a possible mechanism to explain the observed inhibition of HSV replication. However, when we repeated these studies using several known and characterized antioxidants, there was either limited or no inhibitory effects on HSV replication (data not shown). The antioxidants tested included Vitamin E, lycopene, *n*-acetyl-cysteine, glutathione, mercaptoethylamine, pentoxifylline, and pyrrolidineditiocarbamate ammonium. While this does not exclude the possibility that the antioxidant

properties of resveratrol are responsible for the observed results, they are not strongly supportive either.

We next focused our attention on our observation that resveratrol retarded the cell cycle in S–G_2–M, prevented viral reactivation from latency, and had to be added early in the infection process rather than later in order to be effective as an antiviral compound. Collectively, these results suggested that a critical, essential event early in the viral replication scheme, that had a compensating cellular counterpart, was being adversely affected. This possibility gains support from our studies that showed that ICP-4, an essential immediate early regulatory viral protein that is necessary for the transcriptional activation of most of the essential early and late genes, was synthesized in limited quantities in the presence of resveratrol. ICP-4 is one of the earliest proteins produced, and in its absence, HSV replication is defective (Dixon and Schaffer, 1980). Its synthesis is dependent on viral protein VP-16 and cellular proteins Oct-1, HCF and cyclin-dependent kinases cdk-1 and cdk-2 (O'Hare, 1993; Schang et al., 1998). In addition, viral ribonucleotide reductase, which is needed for optimal HSV neurovirulence and reactivation from latency (Jacobsen et al., 1989; Idowa et al., 1992), is required for maximal expression of ICP-4. Viral ribonucleotide reductase is a heterodimeric, multifunctional enzyme that not only reduces ribonucleotides to deoxyribonucleotides for DNA synthesis but also has protein kinase activity on the large subunit. Indeed, it is the protein kinase activity on the large subunit, which is an immediate early protein, that appears to be required for efficient expression of ICP-4 (Smith et al., 1998). Notably, resveratrol is reported to be a potent and specific inhibitor of cellular ribonucleotide reductase (Fontecave et al., 1998) that can also reversibly block the cell cycle in the S/G_2 phase (Ragione et al., 1998).

Since resveratrol is able to effectively inhibit cell ribonucleotide reductase activity, it may also adversely affect viral ribonucleotide reductase and associated kinase activity in the process, reducing the optimal expression of ICP-4. This would, in turn, prevent the synthesis of essential early viral proteins needed for replication or reactivation

from latency (Bates and DeLuca, 1998). Since neither cell or viral ribonucleotide reductase would be available to generate deoxyribonucleotides for DNA synthesis, the cell would arrest in the S/G_2 phase and HSV replication would cease. In this regard, it has been shown that HSV mutants that do not express active ribonucleotide reductase will replicate in growing cells, but not growth arrested G_0/G_1 cells (Goldstein and Weller, 1988). Presumably, the mutant virus utilizes cellular ribonucleotide reductase in growing cells to compensate for its defect in ability to produce active viral ribonucleotide reductase.

While ribonucleotide reductase presents a potential target for resveratrol, we cannot discount the possibility that resveratrol directly lowers ICP-4 production resulting in reduced viral yields. Additionally, resveratrol may act indirectly on viral replication by affecting cellular kinases in the late G_1/S phase of the cell cycle required for efficient HSV replication (Schang et al., 1998). Resveratrol, which has also been shown to inhibit DNA polymerase activity (Sun et al., 1998), could target this viral enzyme limiting viral DNA synthesis, ultimately limiting the production of new progeny.

To determine if other stilbene compounds could adversely affect HSV replication, we tested piceatanol. Piceatanol, a tetrahydroxystilbene, differs from resveratrol, a trihydroxystilbene, in that it has two hydroxy groups on each phenyl ring instead of two on one ring and one on the other. However, when piceatanol was examined, it was found to have no effect on HSV replication. Because piceatanol and resveratrol differ by only one hydroxyl group the observed inhibiting activity of resveratrol on HSV replication suggests a high degree of specificity. This data suggests that there is a structural requirement for the inhibition of HSV replication by stilbene compounds. However, before conclusions can be drawn, more stilbene compounds with similar structure, but variable side groups, will need to be examined.

Of concern throughout these studies was the possible toxicity of resveratrol for the cell culture used in the replication studies of HSV even though it is reported to be a relatively non-toxic compound and a common constituent of the human diet (Goldberg et al., 1995). If resveratrol was toxic in our system, then the cells could not support the replication of HSV accounting for the observed inhibitory effects of resveratrol on HSV replication. Therefore, we examined the effects of this chemical on Vero cells using the trypan blue dye exclusion method. These studies showed that at 50 μg/ml resveratrol had no effect on cell viability but at 100 μg/ml there was an 18% decrease in viable cells at 24 h. All studies were therefore carried out at 50 μg/ml. In support of the trypan blue viability studies were the reversal studies in which HSV replicated in an apparent normal manner when resverstrol was removed at 24 and 48 h after infection. These results suggest that at least those components, both cellular and viral, required for HSV replication were intact.

These results are the first to describe the inhibitory effects of resveratrol, a natural product of certain foods, on HSV replication. The novel nature of stilbenes, their low toxicity, and their ready availability makes them an attractive addition to existing anti-herpetic drugs for further investigation and refinement.

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