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Short communication

Inhibitory activity of oxyresveratrol on wild-type and drug-resistant varicella-zoster virus replication in vitro

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ABSTRACT

The anti-herpes simplex virus (HSV) compound, oxyresveratrol, purified from a Thai traditional medicinal plant *of Artocarpus lakoocha*, was evaluated for its anti-varicella-zoster virus (VZV) activity. This compound exhibited IC_{50} values (50%-inhibitory concentrations for virus plaque formation) of 12.82, 12.80, 12.99 and 12.82 μg/ml against wild type, thymidine kinase-deficient and two types of DNA polymerase mutants with acyclovir-resistance, respectively. Thus oxyresveratrol showed a broad spectrum of anti-VZV activity with a mechanism of action different from that of acyclovir.

Varicella (chicken pox), the primary infection with varicella-zoster virus (VZV), is characterized by fever, vesicular rash, viremia and the virus entering in the nerve ganglia (Gilden et al., 2001). The reactivation of VZV results in herpes zoster (shingles) that causes acute pain in the area innervated by the latently infected ganglia. VZV infections are mainly treated with acyclovir (ACV), valaciclovir, penciclovir, famciclovir or foscarnet (Biron and Elion, 1980; Boyd et al., 1993; Stankus et al., 2000). Chronic VZV infections in immunocompromised hosts require long-term treatment, and ACV-resistant VZV strains have emerged (Pahwa et al., 1988; Hoppenjans et al., 1990; Jacobson et al., 1990; Linnemann et al., 1990; Safrin et al., 1991; Talarico et al., 1993; Boivin et al., 1994; Lyall et al., 1994; Snoeck et al., 1994; Fillet et al., 1998). Similarly to herpes simplex virus (HSV), most of the ACV-resistant VZV strains isolated from patients is thymidine kinase (TK)-deficient (Linnemann et al., 1990; Snoeck et al., 1994; Talarico et al., 1993; Morfin et al., 1999) and these mutants are usually cross-resistant to all other drugs depending on viral TK activity. Foscarnet has been shown to be an alternative treatment and the resistance to foscarnet associated with mutations in the VZV DNA polymerase gene has been described in immunocompromised patients (Fillet et al., 1995; Visse et al., 1998). Since a large number of people are susceptible to the morbidity of VZV, an effective treatment is desirable. In this study, the effects of oxyresveratrol on the replication of wild-type VZV, TK-deficient and DNA polymerase mutants with acyclovir-resistance were examined.

The inhibitory activities of oxyresveratrol (*trans*-2,4,3′,5′-tetrahydroxystilbene) (Fig. 1) on the replication of HSV-1, HSV-2, clinical isolates, thymidine kinase (TK)-deficient and phosphonoacetic acid (PAA)-resistant strains of HSV-1 and its inhibitory activity in HSV-1 mouse cutaneous infection were reported (Chuanasa et al., 2008). Oxyresveratrol was shown to exhibit anti-HSV activity in the plaque reduction assay against wild-type HSV-1 (IC_{50}: 19.8 ± 3.3 μg/ml). The TK-deficient (ACV-resistant) and phosphonoacetic acid (PAA)-resistant HSV-1 strains as well as HSV-2 were susceptible to oxyresveratrol in vitro, similarly to wild-type HSV-1. Its antiviral activity involved a mode of action different from that of ACV as indicated by the synergistic anti-HSV-1 activity with ACV and the susceptibility of ACV-resistant viruses to oxyresveratrol in Vero cells. It allowed early protein synthesis but inhibited late viral protein synthesis of HSV-1 at a concentration of 30 μg/ml. In HSV-1 mouse cutaneous infection, the topical treatment of 30% oxyresveratrol with 1 h before and five times daily after infection showed similar efficacy as 5% ACV cream in delaying the skin lesion development and in protection against death with no significant difference ($P > 0.05$, ANOVA). In addition, oxyresveratrol, orally

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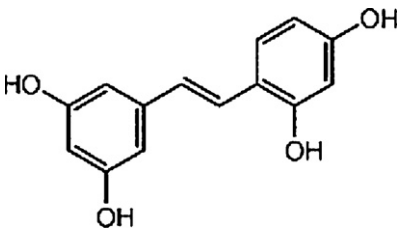

Fig. 1. Structural formula of oxyresveratrol.

administered, at a 500 mg/kg/dose significantly delayed ($P < 0.05$) the development and progression of skin lesions and showed no significant difference in the survival time and percent mortality (Chuanasa et al., 2008). Resveratrol (3,5,4′-trihydroxystilbene), the representative of stilbene group, was previously reported to inhibit HSV and VZV replication in vitro (Docherty et al., 1999, 2006). It exhibited therapeutic activities in HSV cutaneous infection and in vaginal infection in mice (Docherty et al., 2004, 2005). As oxyresveratrol can be abundantly obtained from the heartwood of *Artocarpus lakoocha*, a Thai medicinal plant widely distributed in the country, the anti-VZV activity of oxyresveratrol was evaluated.

In order to determine the anti-VZV activity of oxyresveratrol, the plaque reduction assay was performed (Shiraki et al., 1983, 1992). Human embryonic lung (HEL) cells were grown and maintained in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) for growth and 2% FBS for maintenance. We used the VZV parent Kawaguchi strain, TK-deficient and two types of DNA polymerase mutants with ACV-resistance: A3 with G805C mutation in the DNA polymerase gene (resistant to ACV and phosphonoacetic acid (PAA), sensitive to 9-β-D-arabinofuranosyladenine (Ara-A) and hypersensitive to aphidicolin (Aph)) and A6 with N779S mutation in the DNA polymerase gene (resistant to ACV, sensitive to Aph and hypersensitive to PAA and Ara-A) (Shiraki et al., 1983; Ida et al., 1999; Kamiyama et al., 2001). The anti-VZV activities to parent and mutant strains of acyclovir and foscarnet were determined as well. Briefly, confluent monolayers of HEL cells in 60 mm plastic Petri dishes (in duplicate) were inoculated with 100 plaque forming units per dish of cell-free virus in 0.2 ml SPGC medium (PBS supplemented with 5% sucrose, 0.1% sodium glutamate and 10% FBS). After 1 h of incubation for viral adsorption, oxyresveratrol, dissolved in DMSO and diluted in maintenance medium at a final concentration of 0.1% DMSO, and the drugs, dissolved and diluted in maintenance medium, were added. After 5 days of incubation, the cells were fixed, stained and the plaques were counted. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that inhibited plaque formation by 50%. The cytotoxicity of oxyresveratrol on HEL cells determined by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) reduction assay was also performed. Oxyresveratrol was purified from the heartwood of *A. lakoocha* Roxburgh (Moraceae), a Thai traditional medicinal plant, as previously reported by Sritulaluk et al. (1998) and Likhitwitayawuid et al. (2005). Briefly, the purified compound was analyzed as oxyresveratrol by spectroscopy. The chemical was assayed by HPLC and found to be pure with greater than 99%.

As shown in Table 1, all three VZV mutants were as susceptible to oxyresveratrol as wild type. The IC_{50} values of oxyresveratrol for wild type, TK-deficient and two types of DNA polymerase mutants with ACV-resistance (A3 and A6) were 12.82, 12.80, 12.99 and 12.82 μg/ml, respectively. There were no significant differences among the IC_{50} values ($P > 0.05$ by Student's *t*-test) of oxyresveratrol against wild-type VZV and various VZV mutants. The IC_{50}s of ACV and foscarnet against wild-type VZV were 0.78 ± 0.06 and 6.82 ± 0.85 μg/ml, respectively. It was indicated that all three

Table 1
Anti-VZV activity of oxyresveratrol determined by the plaque reduction assay.

VZV strains	IC_{50}[a] (μg/ml)		
	Acyclovir	Foscarnet	Oxyresveratrol
Wild-type (Kawaguchi)	0.78 ± 0.06	6.82 ± 0.85	12.82 ± 0.27
TK-deficient	>10	6.91 ± 1.46	12.80 ± 0.96
DNA polymerase mutant A3	>10	32.26 ± 5.14	12.99 ± 0.69
DNA polymerase mutant A6	>10	1.77 ± 0.23	12.82 ± 0.21

[a] The IC_{50} (50% inhibitory concentration) was expressed as the mean (μg/ml) ± S.D. for three independent experiments.

mutants tested (TK-deficient, DNA polymerase A3 and DNA polymerase A6 mutants) were ACV-resistant with an $IC_{50} > 10$ μg/ml and only one mutant (DNA polymerase A3 mutant) was foscarnet-resistant with IC_{50} of 32.26 ± 5.14 μg/ml. The DNA polymerase A6 mutant was indicated to be more sensitive to foscarnet (with an IC_{50} of 1.77 ± 0.23 μg/ml than the parent Kawaguchi strain). All VZV mutants were reported to be resistant to ACV and susceptible to phosphonoacetic acid, vidarabine and aphidicolin, at levels similar to those seen with the respective HSV-1 mutants (Shiraki et al., 1983; Kamiyama et al., 2001). As previously reported, oxyresveratrol enhanced the anti-HSV-1 activity of ACV synergistically in Vero cells analyzed by the isobologram method (Chuanasa et al., 2008). This indicated that the mode of anti-VZV activity of oxyresveratrol was different from that of ACV. The 50% cytotoxic concentration of oxyresveratrol on HEL cells was 65 μg/ml as determined by the MTS reduction assay.

In conclusion, oxyresveratrol proved to be inhibitory to wild-type VZV, TK-deficient VZV and DNA polymerase VZV mutants with ACV-resistance. Therefore, oxyresveratrol might be a potential anti-VZV drug candidate for the treatment of VZV infections.

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