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Comparative inhibitory activity of the stilbenes resveratrol and oxyresveratrol on African swine fever virus replication

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ABSTRACT

Stilbenols are polyphenolic phytoalexins produced by plants in response to biotic or abiotic stress. These compounds have received much attention because of their significant biological effects. One of these is their antiviral action, which has previously been documented for two members of this class, namely resveratrol and oxyresveratrol. Here we tested the antiviral effect of these two compounds on African swine fever virus, the only member of the newly created family *Asfarviridae* and a serious limitation to porcine production worldwide. Our results show a potent, dose-dependent antiviral effect of resveratrol and oxyresveratrol *in vitro*. Interestingly, this antiviral activity was found for these synthetic compounds and also for oxyresveratrol extracted from new natural sources (mulberry twigs). The antiviral effect of these two drugs was demonstrated at concentrations that do not induce cytotoxicity in cultured cells. Moreover, these antivirals achieved a 98–100% reduction in viral titers. Both compounds allowed early protein synthesis but inhibited viral DNA replication, late viral protein synthesis and viral factory formation.

1. Introduction

Natural trans polyphenolic stilbenes or stilbenols are products of secondary metabolism that are produced by plants in response to biotic and abiotic stress. Resveratrol, piceatannol and oxyresveratrol are the main representatives of this group of compounds. Stilbenes have numerous remarkable biological properties, pointing to their potential as therapeutic agents in human health. The most known stilbene is resveratrol (trans-3,5,4′-trimethoxystilbene), which is present in the skin of grape berries and in more than 70 other plants (Langcake and Pryce, 1976). The most frequent commercial source of this compound is found in the roots of *Polygonum cuspidatum*, a plant that has been used in traditional Chinese medicine for centuries. Resveratrol is claimed to protect against cancer, heart disease, neurodegenerative disease and inflammation. In addition, it exerts free radical scavenging, antiviral and antioxidant activity (Baur and Sinclair, 2006; Cucciolla et al., 2007; Marques et al., 2009).

Another relevant stilbene is oxyresveratrol (trans-2,3′,4,5′-tetramethoxystilbene). This hydroxylated analog of resveratrol with high structural and biological similarity is present mainly in *Morus alba* bark (Shin et al., 1998) as well as in a few other plants (Sritulakuk et al., 1998; Ban et al., 2006). However, as the natural sources of oxyresveratrol are much more limited than those of resveratrol, its effects have not been as extensively addressed.

Oxyresveratrol is a potent antioxidant and free radical scavenger (Lorenz et al., 2003), and an effective tyrosinase inhibitor (Sritulakuk et al., 1998; Shin et al., 1998; Li et al., 2007). The most documented biological effect of oxyresveratrol is in the field of neuroprotection (Andrabi et al., 2004; Ban et al., 2006; Chao et al., 2008).

One of the many biological effects of these stilbenols is their antiviral action. Resveratrol shows potent antiviral activity against various families of DNA and RNA viruses and it seems that this compound interferes with viral infection by altering cellular pathways rather than by acting directly against the virus itself (Campagna and Rivas, 2010). The first report on resveratrol was published in 1999. The authors showed that the addition of this drug in the first 6 h of herpes simplex virus (HSV), HSV-1 and HSV-2 infection blocked viral replication in a dose-dependent manner (Docherty et al., 1999). Other members of the *Herpes viridae* family are also susceptible to resveratrol treatment. Moreover, replication of VZV (varicella-zoster virus), HCMV (human cytomegalovirus) and EBV (Epstein–Barr virus) is inhibited by resveratrol in a dose-dependent manner (Docherty et al., 2006; Evers et al., 2004; Kapadia et al., 2002). This drug also inhibits polyomavirus replication by blocking the synthesis of viral DNA *in vitro* (Berardi et al., 2009). It has also been shown to exert strong antiviral activity against the influenza virus *in vitro* and *in vivo* by inducing a decrease in the translation of late viral proteins (Palamara et al., 2005).

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Several authors have analyzed the effect of resveratrol on HIV-1 (human immunodeficiency virus-1) infection and various mechanisms of action have been proposed (Heredia et al., 2000; Krishnan and Zeichner, 2004; Wang et al., 2004; Zhang et al., 2009). Recent publications have demonstrated that resveratrol inhibits the growth of vaccinia virus (Cheltsov et al., 2010). Nevertheless, in contrast to its antiviral activity against other viruses, resveratrol enhances HCV (hepatitis C virus) replication (Nakamura et al., 2010).

Oxyresveratrol also shows antiviral activity. The inhibitory activity of this drug in HSV-1 (Chuanasa et al., 2008) and in VZV infections (Sasivimolphan et al., 2009) has been well documented.

African swine fever virus (ASFV) is a large enveloped DNA virus with a genomic composition similar to that of poxviruses, although the virion structure and morphology resemble those of iridoviruses (Dixon et al., 2000). It causes an acute haemorrhagic fever in domestic pigs, causing mortality rates approaching 100%. Unfortunately, no effective vaccine against ASFV is available. Consequently, the development of new antiviral agents against this devastating virus is crucial.

Here we studied the effect of the stilbenols resveratrol and oxyresveratrol on the replication of ASFV. Given the limited availability of oxyresveratrol on the market, we used two protocols to obtain the compound, namely extraction from *M. alba* twigs, and chemical synthesis. The efficiency of these approaches was compared. We then analyzed the capacity of resveratrol and oxyresveratrol to protect Vero cells from ASFV infection.

2. Material and methods

2.1. Source of stilbenols

The oxyresveratrol used was obtained by two protocols. One was based on the isolation from *M. alba* twigs, and the other by chemical synthesis of the compound.

The isolation procedure was performed as described previously (Li et al., 2007). Briefly, 200 g powdered dried mulberry twigs was extracted with methanol. After combination and concentration, about 7 g of dried extract was dissolved in deionized water. It was subsequently successively partitioned with chloroform, ethyl acetate, and n-butanol. The ethyl acetate fraction (3.5 g) was further isolated on a Sephadex LH-20 column and silica gel column to obtain pure oxyresveratrol.

Oxyresveratrol was also prepared by standard synthetic procedures, as outlined in Fig. 1, starting from the commercially available 3,5-dimethoxybenzyl bromide (2), which was efficiently converted into the corresponding 3 phosphonate by a Michael–Arbuzov reaction with triethyl phosphite. Subsequently, a Horner–Emmons–Wadsworth reaction of the 3 phosphonate with 2,4-dimethoxybenzaldehyde led to an 80% yield of the mixture of stilbenes 4 but with modest trans selectivity (75:25). After treatment with iodine, the cis isomer was fully converted into the stilbene with the desired trans geometry E-4. Finally, (E)-3,5,2′,4′-tetramethoxystilbene (E-4) was demethoxylated by thermal treatment with methylmagnesium iodide, providing the tetraol 1 in a 21% yield. This compound showed identical spectroscopic data to those shown by a natural sample of oxyresveratrol isolated from mulberry twigs using the above described protocol and purities of 96–98% were obtained with both preparation procedures. Our data matched those reported for this compound (Choi et al., 2006).

2.2. Cell culture and viruses

Vero (African green monkey kidney) cell lines were obtained from the American Type Culture Collection (ATCC) and grown and maintained at 37 °C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% calf fetal serum, 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine.

ASFV isolate BA71V was used. In fluorescence experiments we used an infectious recombinant ASFV, B54GFP-2, which expresses and incorporates into the virus particle a chimera of the p54 envelope protein fused to the enhanced green fluorescent protein (EGFP) (Hernáez et al., 2006). Preparation of viral stocks, titrations, and infection experiments were carried out in cells as previously described (Enjuanes et al., 1976).

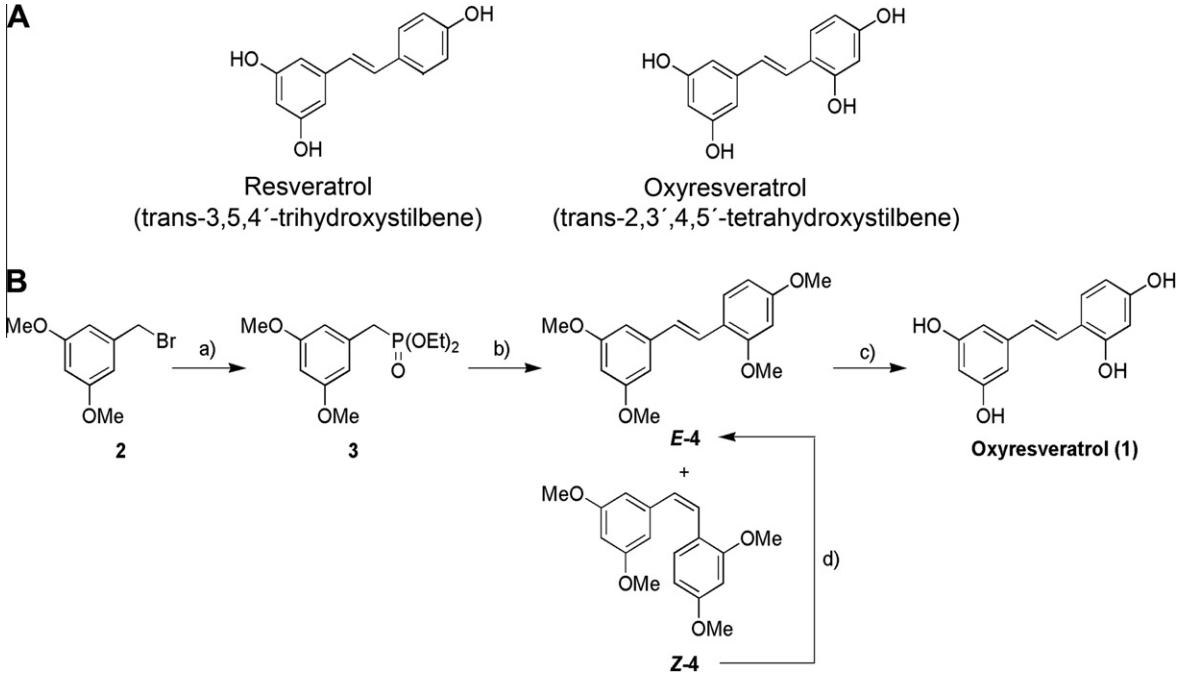

Fig. 1. (A) Structure of Stilbenols. (B) Synthesis of oxyresveratrol (1) Reagents and conditions: (a) $P(OEt)_3$, TBAI, 130 °C; (b) NaH, 2,4-$(MeO)_2C_6H_3CHO$, THF, from 0 °C to room temperature; (c) MeMgI, heat; (d) I_2, heptane, reflux.