



Short communication

Investigation of the influence of EPs[®] 7630, a herbal drug preparation from *Pelargonium sidoides*, on replication of a broad panel of respiratory viruses

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ABSTRACT

The *Pelargonium sidoides* extract EPs[®] 7630 is an approved drug for the treatment of acute bronchitis in Germany. The postulated mechanisms underlying beneficial effects of EPs[®] 7630 in bronchitis patients include immunomodulatory and cytoprotective effects, inhibition of interaction between bacteria and host cells, and increase of ciliary beat frequency on respiratory cells. Here, we investigated the influence of EPs[®] 7630 on replication of a panel of respiratory viruses. Determination of virus-induced cytopathogenic effects and virus titres revealed that EPs[®] 7630 at concentrations up to 100 µg/ml interfered with replication of seasonal influenza A virus strains (H1N1, H3N2), respiratory syncytial virus, human coronavirus, parainfluenza virus, and coxsackie virus but did not affect replication of highly pathogenic avian influenza A virus (H5N1), adenovirus, or rhinovirus. Therefore, antiviral effects may contribute to the beneficial effects exerted by EPs[®] 7630 in acute bronchitis patients.

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Introduction

In southern Africa, the roots of *Pelargonium sidoides* have been used for centuries for the treatment of different ailments including infections of the airways (Conrad et al. 2007; Brendler and van Wyk 2008; Kolodziej 2008). In Germany, a standardised extract of *Pelargonium sidoides* (EPs[®] 7630) was registered in 2005 by the Federal Institute for Drugs and Medical Devices (BfArM) for the indication “acute bronchitis” (Conrad et al. 2007). A couple of randomised, double-blind, placebo-controlled clinical trials support the efficacy and safety of EPs[®] 7630 for the treatment of acute bronchitis in adults and children (Conrad et al. 2007; Agbabiaka et al. 2008; Matthys and Funk 2008; Timmer et al. 2008; Kamin et al. 2010a,b; Matthys et al. 2010). The postulated mechanisms underlying beneficial effects of EPs[®] 7630 in bronchitis patients include immunomodulatory and cytoprotective effects, inhibition of interaction between bacteria and host cells, and increase of ciliary beat frequency on respiratory cells (Conrad et al. 2007; Kolodziej 2008).

Notably, bacteria are estimated to cause <10% of cases of uncomplicated acute bronchitis while >90% of cases are estimated to be caused by respiratory viruses (Gonzales and Sande 2000). Therefore, effects of EPs[®] 7630 on replication of respiratory viruses are of interest in the context acute bronchitis. An aqueous extract of *Pelargonium sidoides* had been already tested for antiviral effects against herpes simplex virus type 1 (HSV-1) and 2 (HSV-2)

(Schnitzler et al. 2008) common viruses that cause recurrent orofacial or genital lesions (Cernik et al. 2008). The extract inhibited replication of both viruses through interference with virus surface resulting in virus inactivation (Schnitzler et al. 2008).

Here, we investigated the effects of EPs[®] 7630 on virus replication of a broad panel of respiratory viruses including Influenza A virus (H1N1, H3N2, H5N1), respiratory syncytial virus (RSV), human coronavirus, adenovirus, parainfluenza virus, coxsackie virus, and rhinovirus.

Materials and methods

Drugs

EPs[®] 7630 was provided by the Phytochemistry Section of the Department of Preclinical Research, Dr. Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany).

Cells and viruses

The following viruses were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA): respiratory syncytial virus (RSV) strain A2 (ATCC-No. VR-1540), adenovirus 3 strain GB (adeno 3, ATCC-No. VR-3), adenovirus 7 strain Gomen (adeno 7, ATCC-No. VR-7), parainfluenza virus type 3 strain C243 (parainfluenza 3, ATCC-No. VR-93), human rhinovirus 16 strain 11757 (rhino 16, ATCC-No. VR-283).

The influenza virus strains A/New Caledonia/20/99 (H1N1) and A/California/7/2004 (H3N2) were received from the World

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Table 1

Influence of EPs® 7630 on virus-induced formation of cytopathogenic effects (CPE) and on cell viability.

Virus	Cell	IC ₅₀ ^a (µg/ml)	CC ₅₀ ^b (µg/ml)	TI ^c
H1N1	MDCK	9.45 ± 2.94	>100 µg/ml (114 ± 15%) ^d	>10.6
H3N2	MDCK	8.66 ± 1.06	>100 µg/ml (114 ± 15%)	>11.5
H5N1	Vero	>100	>100 µg/ml (85 ± 10%)	n.d. ^e
RSV	Vero	19.65 ± 1.77	>100 µg/ml (85 ± 10%)	>5.1
HCo-229E	Caco-2	44.50 ± 15.84	>100 µg/ml (86 ± 12%)	>2.3
Adeno 3	Caco-2	>100	>100 µg/ml (86 ± 12%)	n.d.
Adeno 7	Caco-2	>100	>100 µg/ml (86 ± 12%)	n.d.
Parainfluenza 3	LLC-MK2	74.35 ± 17.89	>100 µg/ml (81 ± 14%)	>1.3
Coxsackie A9	HFF	14.80 ± 3.39	>100 µg/ml (85 ± 9%)	>6.8
Rhino 16	Mel-Ho	>100	>100 µg/ml (87 ± 13%)	n.d.

^a Concentration that inhibits CPE formation by 50%.^b Concentration that decreases cell viability by 50%.^c Therapeutic index = CC₅₀/IC₅₀.^d Cell viability in the presence of EPs® 7630 100 µg/ml.^e Not determinable.

Health Organization (WHO) Influenza Centre (National Institute for Medical Research, London, UK). The H5N1 influenza strain A/Thailand/1(Kan-1)/04 was obtained from Prof. Pilaipan Puthavathana (Mahidol University, Bangkok, Thailand). The human coronavirus strain 229E (HCo-229E) was obtained from John Ziebuhr (University of Würzburg, Germany). Coxsackie virus A9 (coxsackie A9) was isolated from one of our patients.

The following cell lines were used for the cultivation of viruses: Madin-Darby canine kidney cell line MDCK (ATCC-No. CCL-34; H3N2, H1N1, influenza B), Vero (ATCC-No. CCL-81; RSV, H5N1), Caco-2 (ATCC-No. HTB-37, adeno 3, adeno 7, HCo-229E), LLC-MK2 (ATCC-No. CCL-7; parainfluenza 3), Mel-Ho (obtained from DSMZ, Braunschweig, Germany, DSMZ-No. ACC 62, rhino 16), human foreskin fibroblasts (HFFs, established as described previously (Cinatl et al. 1994); coxsackie A9). All cells were cultured in MEM supplemented with 10% foetal calf serum (FCS).

Virus stocks were prepared by infecting cells with the respective viruses. Aliquots were stored at –80 °C. Virus titres were determined as 50% tissue culture infectious dose (TCID₅₀/ml) in 96-well microtiter plates. The infectious titre was determined by the method of Spearman and Kärber (Spearman 1908; Kärber 1931). The maintenance medium during infection was for influenza viruses serum-free MEM supplemented with 2 µg/ml trypsin and for all other viruses MEM supplemented with 2% FCS.

Cytopathogenic effect (CPE) reduction assay

The cytopathogenic effect (CPE) reduction assay was performed as described before (Michaelis et al. 2010). Confluent cell monolayers grown in 96-well microtitre plates were infected with respective viruses in the presence or absence of EPs® 7630. The following multiplicities of infection (MOIs) were used: H1N1, 0.005; H3N2, 0.005; influenza B, 0.01; coxsackie A9, 0.005; RSV, 0.01; adeno 3, 0.01; adeno 7, 0.02, HCo-229E, 0.01; parainfluenza 3; 0.01; rhino 16, 0.005; H5N1, 0.001.

The virus-induced CPE were recorded using an inverted light microscope at 24 h (H1N1, H3N2, H5N1, influenza B, coxsackie A9, coxsackie B5), 48 h (coxsackie B4, rhino 16), 72 h (RSV, HCo-229E, parainfluenza 3), or 96 h (adeno 3, adeno 7, parainfluenza 2) post infection (p.i.). EPs® 7630 was continuously present starting with a 1 h pre-incubation period.

Cell viability assay

The effect of EPs® 7630 on cell viability was investigated in non-infected cells that were treated in the same way with EPs® 7630 like the virus-infected cells in the CPE reduction assay. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-

2,5-diphenyltetrazolium bromide (MTT) dye reduction assay as described before (Michaelis et al. 2004).

Virus yield reduction assay

Infectious virus titres were determined as described before at “Cells and viruses”. Cells were infected with the respective viruses in the absence or presence of EPs® 7630 at the following MOIs: H1N1, 0.005 (titres were determined 24 h post infection); H3N2, 0.005 (24 h); coxsackie B5, 0.005 (24 h); coxsackie A9, MOI 0.005 (24 h); parainfluenza 3, 0.01 (72 h); RSV, 0.01 (72 h); HCo-229E, 0.01 (72 h).

Results

Influence of EPs® 7630 on virus-induced cytopathogenic effect (CPE) formation

EPs® 7630 did not decrease viability of all investigated cell types in concentrations up to 100 µg/ml. Moreover, EPs® 7630 did not affect CPE formation induced by H5N1, adeno 3, adeno 7, or rhino 16 in the examined concentrations up to 100 µg/ml. However, EPs® 7630 interfered with CPE formation caused by H1N1, H3N2, RSV, HCo-229E, parainfluenza 3, or coxsackie A9 (Table 1).

Influence of EPs® 7630 on infectious virus titres

Inhibition of virus-induced CPE formation may be a consequence of inhibition of virus replication, of cytoprotective effects without influence on virus replication, or of a combination of both. Therefore, the influence of EPs® 7630 was investigated on H1N1, H3N2, RSV, HCo-229E, parainfluenza 3, or coxsackie A9 virus titres. EPs® 7630 decreased titres of all susceptible viruses in a dose-dependent manner (Fig. 1).

Discussion

Here, we investigated the effect of EPs® 7630 on replication of a panel of respiratory viruses. EPs® 7630 inhibited the replication of the respiratory viruses H1N1, H3N2, RSV, HCo-229E, parainfluenza 3, and coxsackie A9.

The magnitude of EPs® 7630-induced antiviral effects differed between the sensitive viruses. EPs® 7630 at a concentration of 100 µg/ml completely suppressed replication of the seasonal influenza A virus strains (H1N1, H3N2) and of RSV. Virus titres of coxsackie A9 were reduced by about 10,000-fold, of parainfluenza 3 by about 150-fold, and of HCo-229E by about 10-fold. A 10-fold decrease of virus titres may be sufficient to prevent viral disease, especially in a pre-emptive setting.

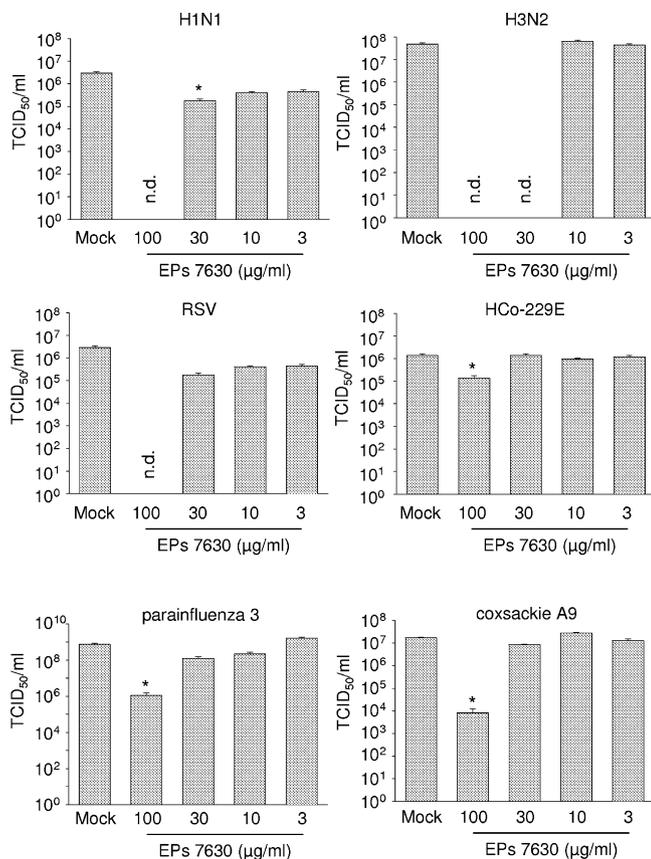


Fig. 1. Influence of EPs[®] 7630 on infectious virus titres indicated as 50% tissue culture infectious dose (TCID₅₀/ml). EPs[®] 7630 was continuously present in cell culture media beginning with a 1 h pre-incubation period. Cells were infected with the respective viruses in the absence (Mock) or presence of EPs[®] 7630 at the following MOIs: influenza A virus H1N1, 0.005 (titres were determined 24 h post infection, cell line MDCK); influenza A virus H3N2, 0.005 (24 h, MDCK); coxsackie virus A9 (coxsackie A9), MOI 0.005 (24 h, human foreskin fibroblasts); parainfluenza virus type 3 (parainfluenza 3), 0.01 (72 h, LLC-MK2); respiratory syncytial virus (RSV), 0.01 (72 h, Vero); human coronavirus strain 229E (HCoV-229E), 0.01 (72 h, Caco-2). **P* < 0.05 relative to Mock (as determined by ANOVA with subsequent Student–Newman–Keuls test); n.d. = not detectable.

All viruses that were inhibited by EPs[®] 7630 are enveloped viruses (H1N1, H3N2, HCoV-229E, RSV, parainfluenza 3) except coxsackie A9 while all non-sensitive viruses were non-enveloped viruses (adeno 3, adeno 7, rhino 16) with the exemption of the highly pathogenic avian influenza A virus H5N1 (Knipe and Howley 2007). Therefore, EPs[®] 7630 appears to primary target enveloped viruses. In accordance, an aqueous extract of *Pelargonium sidoides* had been found to inhibit replication of the enveloped viruses HSV-1 and HSV-2 through interference with the virus surface resulting in virus inactivation (Schnitzler et al. 2008). However, since EPs[®] 7630 inhibited replication of the non-enveloped coxsackie A9 other mechanisms may also play a role.

The varying sensitivities to EPs[®] 7630 of the seasonal influenza A strains H1N1 and H3N2 in comparison to H5N1 cannot be explained by our data. H5N1 viruses are generally known to be less sensitive to antiviral treatment than seasonal strains (Michaelis et al. 2009). Moreover, the influenza A virus haemagglutinin proteins that bind to the sialic acids on the target cell's surface as a first step in the viral infection process significantly vary between the seasonal strains and H5N1 (Michaelis et al. 2009) and may therefore be differently affected by agents that interact with the virus surface.

In conclusion, we show that the *Pelargonium sidoides* extract EPs[®] 7630 that is approved for the treatment of acute bronchitis interferes with replication of different respiratory viruses includ-

ing seasonal influenza A virus strains, RSV, human coronavirus, parainfluenza virus, and coxsackie virus. Since respiratory viruses are estimated to cause >90% of cases of acute bronchitis (Gonzales and Sande 2000) antiviral effects may contribute to the beneficial effects of EPs[®] 7630 described in human acute bronchitis patients (Conrad et al. 2007; Agbabiaka et al. 2008; Matthys and Funk 2008; Timmer et al. 2008; Kamin et al. 2010a,b; Matthys et al. 2010).

Conflict of interest

The work was financially supported by Dr. Willmar Schwabe Pharmaceuticals (Karlsruhe, Germany).

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