Antiviral Research 123 (2015) 15–21

Contents lists available at ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Resveratrol inhibits rhinovirus replication and expression of inflammatory mediators in nasal epithelia

Paola Mastromarino [a,*], Daniela Capobianco [a], Federica Cannata [a], Chiara Nardis [a], Elena Mattia [a], Alessandra De Leo [a], Rossella Restignoli [b], Antonio Francioso [c], Luciana Mosca [c]

[a] Department of Public Health and Infectious Diseases, Section of Microbiology, Sapienza University, I-00185 Rome, Italy
[b] NOOS s.r.l., I-00181 Rome, Italy
[c] Department of Biochemical Sciences, Sapienza University, I-00185 Rome, Italy

ARTICLE INFO

Article history:
Received 15 June 2015
Revised 31 July 2015
Accepted 17 August 2015
Available online 19 August 2015

Keywords:
Resveratrol
Human rhinovirus
Inflammatory mediators
Nasal epithelia

ABSTRACT

Human rhinoviruses (HRV), the cause of common colds, are the most frequent precipitants of acute exacerbation of asthma and chronic obstructive pulmonary disease, as well as causes of other serious respiratory diseases. No vaccine or antiviral agents are available for the prevention or treatment of HRV infection. Resveratrol exerts antiviral effect against different DNA and RNA viruses. The antiviral effect of a new resveratrol formulation containing carboxymethylated glucan was analyzed in H1HeLa cell monolayers and *ex vivo* nasal epithelia infected with HRV-16. Virus yield was evaluated by plaque assay and expression of viral capsid proteins by Western blot. IL-10, IFN-β, IL-6, IL-8 and RANTES levels were evaluated by ELISA assay. ICAM-1 was assessed by Western blot and immunofluorescence. Resveratrol exerted a high, dose-dependent, antiviral activity against HRV-16 replication and reduced virus-induced secretion of IL-6, IL-8 and RANTES to levels similar to that of uninfected nasal epithelia. Basal levels of IL-6 and RANTES were also significantly reduced in uninfected epithelia confirming an anti-inflammatory effect of the compound. HRV-induced expression of ICAM-1 was reversed by resveratrol. Resveratrol may be useful for a therapeutic approach to reduce HRV replication and virus-induced cytokine/chemokine production.

1. Introduction

Human rhinoviruses (HRV) – the most prevalent respiratory viruses – are responsible for at least 50% of the common colds (Makela et al., 1998; Rollinger and Schmidtke, 2011) a mild, self-limiting upper respiratory tract illness, nonetheless with major economic impact through loss of productivity (Fendrick et al., 2003). HRV infection is also associated with acute otitis media, sinusitis, bronchiolitis, pneumonia, and severe infections, especially in immunocompromised patients (Henquell et al., 2012; Lieberman et al., 2010; Ruuskanen et al., 2013). HRV are also the major cause of exacerbations in both chronic obstructive pulmonary disease (COPD) and asthma (Busse et al., 2010; Papi et al., 2006).

HRV are positive-sense RNA viruses of the *Picornaviridae* family. The coding region of the viral genome contains sequences for non-structural and structural proteins divided into three primary precursor molecules (P1, P2, and P3). The four structural proteins (VP1, VP2, VP3 and VP4), which form the viral capsid, are derived from the P1 portion of the polyprotein by sequential proteolytic cleavages (Bedard and Semler, 2004).

There are no approved antiviral agents for the prevention or treatment of HRV infection. Several drug candidates have been progressed into clinical trials, including a viral 3C protease inhibitor, and different compounds that prevent virus attachment and entry into cells by binding to the viral capsid (Gunawardana et al., 2014). However, none of these candidates were commercialized due to unacceptable side effects or lack of efficacy when applied to the natural setting (Docherty et al., 2005). Currently, capsid binder vapendavir is under clinical development (Feil et al., 2012). Prevention of HRV infection through vaccination is not feasible since more than 100 different rhinovirus types with

Abbreviations: HRV, human rhinovirus; COPD, chronic obstructive pulmonary disease; CMG, carboxymethylated-(1,3/1,6)-β-D-glucan.

* Corresponding author at: Dipartimento di Sanità Pubblica e Malattie Infettive, Sezione di Microbiologia, Università "Sapienza", Piazzale Aldo Moro 5, I-00185 Roma, Italy.

E-mail address: paola.mastromarino@uniroma1.it (P. Mastromarino).

http://dx.doi.org/10.1016/j.antiviral.2015.08.010
0166-3542/

low antibody cross-reactivity have been described (Papi and Contoli, 2011).

Resveratrol is a polyphenol produced by several plants in response to stress or injury induced by microorganisms or environmental hazards and protects fruits and vegetables against fungal infections (Pervaiz, 2003; Sanders et al., 2000). Resveratrol displays a wide range of biological and pharmacological activities including anti-inflammatory, antioxidative, anticancer, antibacterial, antiviral, cardioprotective and neuroprotective effects (Campagna and Rivas, 2010; Chan, 2002; Fremont, 2000; Pervaiz, 2003). However, in spite of its multiple beneficial effects on human health, resveratrol use as a drug has been limited by its poor solubility, low bioavailability, and the tendency to become unstable due to autooxidation and photosensitivity (Francioso et al., 2014a). A recent paper describes the development of an aqueous formulation of resveratrol in combination with a modified glucan, the carboxymethylated-(1,3/1,6)-β-D-glucan (CMG) (Francioso et al., 2014b). CMG confers stability to resveratrol in solution without affecting its biological activity. This new formulation provided as a nasal spray was recently proved to be safe and effective in reducing the severity and recurrence of respiratory infections in children (Miraglia Del Giudice et al., 2014a,b; Varricchio et al., 2014).

Several studies have demonstrated that resveratrol exerts antiviral effect against different DNA and RNA viruses, including herpes simplex virus, human cytomegalovirus, varicella-zoster virus, Epstein Barr virus, influenza A virus, respiratory syncytial virus, and HIV both *in vitro* and *in vivo* (Beach et al., 2014; Chen et al., 2012; De Leo et al., 2012; Docherty et al., 2005, 2006; Evers et al., 2004; Faith et al., 2006; Lin et al., 2015; Palamara et al., 2005; Xie et al., 2012).

The benefits of resveratrol may be associated with its general activity as a modulator of the transcription factor NF-kB, of the cell cycle, apoptosis, and possibly as activator of SIRT1 (Campagna and Rivas, 2010). NF-κB transcription mediates the production of cytokines and chemokines in response to toll-like receptor recognition of intermediate dsRNA during viral infections (Majde, 2000; Rudd et al., 2006).

Human airway epithelial cells (HAECs) are the principal sites of HRV infection in both upper and lower airways (Holgate, 2011). HAECs not only serve as a target and possible reservoir for the infecting virus, but also are the site and source of a wide range of mediators that drive subsequent immune and physiological responses specific to HRV (Holgate, 2013). Indeed, rhinovirus infection induces the production of cytokines and chemokines including interleukin-6 (IL-6), interleukin-8 (IL-8), regulated on activation normal T cell expressed and secreted (RANTES), interleukin-10 (IL-10) and interferon-β *in vivo* and *in vitro* (Bartlett et al., 2008; Message et al., 2008; Subauste et al., 1995; Zhu et al., 1996). The production of most cytokines and chemokines is HRV replication dependent (Bartlett et al., 2008), has pro-inflammatory effects and correlates with the severity of cold symptoms (Gern et al., 2002). Moreover, rhinovirus infection of both primary bronchial epithelial cells and a respiratory epithelial cell line markedly increases cell surface expression of intercellular adhesion molecule-1 (ICAM-1) (Papi and Johnston, 1999) the cellular receptor for the major group (90%) of rhinoviruses (Greve et al., 1989; Uncapher et al., 1991).

To date, no studies investigated the effects of resveratrol on inflammatory mediator production in HRV-infected airway epithelia. In this study, we examined the effect of resveratrol on chemokine and cytokine production in HRV-infected nasal epithelia *ex vivo*, and evaluated the ability of resveratrol to suppress production of infectious virus in nasal epithelia and in H1HeLa cells. Nasal epithelia were cultured at air–liquid interface using a two-chamber trans-well tissue culture system to reproduce the structural and functional phenotype of differentiated airway epithelium. Resveratrol was tested alone or in combination with CMG to examine possible synergistic activity of the compounds. The effect of resveratrol on the production of ICAM-1 in both cell systems was also analyzed.

2. Materials and methods

2.1. HeLa cells

H1HeLa cells, particularly susceptible to rhinovirus infection, were used. Cells were cultured at 37 °C in 5% CO_2 atmosphere in Eagle's Minimum Essential Medium (MEM, HyClone) with 10% fetal bovine serum (FBS). For viral infection the serum concentration was lowered to 2% (maintenance MEM).

2.2. Human nasal epithelia

Fully differentiated human nasal epithelial models reconstituted using primary nasal cells isolated from healthy donors (MucilAir™ Epithelix Sarl) were cultured on a Transwell of 6.5 mm of diameter, with a pore size of 0.4 μm (Costar) at the air–liquid interface, using MucilAir™ culture medium at 37 °C in 5% CO_2 atmosphere. Each insert, consisting of about 400,000 cells, mimics the tissue of the human nasal epithelium and contains, from the bottom to the top, basal, goblet, ciliated cells and mucus.

2.3. Virus

Rhinovirus serotype 16 (HRV-16, ATCC VR-283) was grown in H1HeLa cells inoculated with virus at a multiplicity of infection (MOI) of 0.1 PFU/cell in maintenance medium.

2.4. Plaque assay

Plaque assay was performed on H1HeLa cells inoculated with ten-fold serial dilutions of HRV-16. After 48 h of incubation at 33 °C, plaques were stained with 0.1% crystal violet solution.

2.5. Cytotoxicity assays

Non cytotoxic concentrations of CMG or resveratrol for H1HeLa cells and nasal epithelia were determined by MTT and resazurin assay, respectively. See Supplementary Materials.

2.6. Antiviral assays

The antiviral effect of resveratrol and/or CMG in H1HeLa cells was evaluated before, during and after viral adsorption. Antiviral effects of the compounds were also evaluated in human nasal epithelia. HRV-16 infection was performed on the apical side of epithelia. After 7 h viral adsorption at 37 °C in 5% CO_2 atmosphere, cells were washed 3 times with MucilAir™ culture medium to remove unabsorbed virus. The compounds were then added in the culture medium and on the apical surface (30 μL). Cells from duplicate wells were scraped with cold PBS, centrifuged at 400*g* for 5 min at 4 °C and resuspended in Laemmli sample buffer for Western blot analysis.

2.7. Western blot analysis

Cell extracts prepared as above described were resolved on 8% SDS-polyacrylamide gels. After blotting, membranes were probed with ICAM-1 (1:4000; Santa Cruz), VP2 (1:8000; QED Bioscience