Summary. Resveratrol, a natural polyphenolic molecule with several biological activities, is a well recognized anti-oxidant, anti-aging and cancer chemopreventive agent. Moreover, resveratrol anti-inflammatory and antifibrotic properties have been demonstrated both in vitro and in different animal models of inflammatory pathologies, including bowel and liver diseases. We review the evidence of resveratrol protective role in respiratory diseases such as acute lung injury, asthma, chronic obstructive pulmonary disease and lung fibrosis. We conclude that resveratrol and its derivatives may act as a therapeutic agents in respiratory diseases and pertinent clinical trials should be performed.

Key words: Acute lung injury, Asthma, COPD, Fibrosis

Introduction

Resveratrol (3,5,4-trihydroxystilbene), is a non-flavonoid polyphenolic molecule found in a number of different plant species, mainly in skin of grapes, peanuts, soy beans, pomegranates, bilberry, mulberries and also in red wine (from grape skins). Resveratrol is present in cis/trans isoforms (both isomers may be glucosylated) and the major trans isomer is the biologically active one; it has been reported to exert a number of health benefits such as antioxidant (Farris et al., 2013), anti-inflammatory (de la Lastra and Villegas, 2005; Das and Das, 2007; Svajger and Jeras, 2012), antiviral (Heredia et al., 2000; Campagna and Rivas, 2010; Galindo et al., 2011; Clouser et al., 2012; Xie et al., 2012; Xu et al., 2013; Singh and Pai, 2014), and anticancer activities (Jang et al., 1997; Heredia et al., 2000; Granados-Soto, 2003; Aggarwal et al., 2004; Athar et al., 2007; Wood et al., 2010; Aluyen et al., 2012; Svajger and Jeras, 2012; Yang et al., 2014), through many different mechanisms. Resveratrol has also been suggested to underlay the beneficial properties of red wine and thus to be a major contributor at the 'French paradox', i.e. lower mortality due to cardiovascular diseases (CVD) in people living in certain parts of France (habitual red wine consumers), in comparison with other Western countries sharing CVD risk factors (St Leger et al., 1979; Renaud and de Lorgeril, 1992; Catalgol et al., 2012). Actually, epidemiological studies have demonstrated that red wine consumption may improve endothelial function and reduce the risk of cardiovascular diseases (Gronbaek et al., 2001; Cordova et al., 2005; Opie and Lecour, 2007). Moreover, a protective role of resveratrol has been widely showed in animal models of cardiovascular diseases (Hung et al., 2004; Fukao et al., 2004; WHO 2004; Wang et al., 2005; Burstein et al., 2007; Lamont et al., 2011; Robich et al., 2012).

A positive association of resveratrol intake with lung function has been observed in the general population (Siedlinski et al., 2012) and there is increasing evidence for a protective role of resveratrol in respiratory diseases (Wood et al., 2010). We review anti-inflammatory and antifibrotic effects of resveratrol in lung diseases.

Acute lung injury (ALI)

ALI, a life-threatening syndrome causing high morbidity and mortality worldwide, is characterized by
non-cardiogenic, acute and progressive respiratory failure induced by a variety of injurious stimuli (Walkey et al., 2012). The development of ALI during sepsis almost doubles the mortality rate of patients. Resveratrol was shown to exert a protective effect against ALI induced by lipopolysaccharide (LPS), by inhibiting the myd88-dependent TLR4 signaling pathway (Zhang et al., 2014c), via activation of sirtuin 1 (Sirt1) (Li et al., 2013) and markedly decreasing the production of inflammatory cytokines, such as IL-1β and MIP-1α, as well as preventing the release of nitric oxide (NO) by inhibiting the expression of inducible NO synthase besides suppressing the nuclear translocation of NF-κB in the lung tissue (Cao et al., 2011).

A protective effect of resveratrol was also shown in endotoxemia-induced lung injury through the reduction of oxidative/nitrative stress (Zhang et al., 2014a). Moreover, severe acute pancreatitis-associated lung injury in rats was shown to be effectively attenuated by resveratrol (Wang et al., 2014), via inhibition of apoptosis (Sha et al., 2009) as well as by reducing the intracellular calcium overload (Wang et al., 2008), and improving the microcirculation disorder of the lung by several mechanisms (Meng et al., 2005). Resveratrol was also shown to attenuate the half sulfur mustard gas-induced acute lung injury in rats (McClintock et al., 2002, 2006). Furthermore, 3,5,4′-tri-O-acetylresveratrol was demonstrated to ameliorate seawater exposure-induced lung injury by upregulating connexin 43 expression (Ma et al., 2013a) and inhibiting activation of nuclear factor-kappa B and hypoxia-inducible factor-1α (Ma et al., 2013b).

Multiple anti-inflammatory pathways triggered by resveratrol were shown to ameliorate staphylococcal enterotoxin B-induced lung injury (Rieder et al., 2012).

**Asthma**

Asthma is a chronic inflammatory disorder of the airways characterized by three interrelated features: 1) airway inflammation in which many cells and elements are involved in the activation of both the acquired and innate immune systems; 2) airway hyperresponsiveness (AHR, easily triggered bronchospasm); 3) airway remodeling with reversible airflow obstruction (Kudo et al., 2013). Resveratrol and SRT1720, a synthetic activator of Sirt1, were recently shown to exert a beneficial role in the ovalbumin (OVA)-induced asthma mouse model which displays most of the clinicopathological features of severe human asthma (Ichikawa et al., 2013). In a previous study it was demonstrated that resveratrol attenuates the asthma phenotype in the same mouse experimental model of chronic allergic asthma by restoring inositol polyphosphate 4 phosphatase (INPP4A) and down-regulating the PI3K-Akt pathway (Aich et al., 2012). Moreover, in that model it was also demonstrated that treatment with resveratrol can simultaneously inhibit airway inflammation and structural remodeling changes which are the main contributors to AHR and irreversible lung function loss in asthma (Royce et al., 2011). Furthermore, resveratrol was shown to significantly inhibit increases in T-helper-2-type cytokines such as IL-4 and IL-5 in plasma and bronchoalveolar lavage fluid (BALF), as well as to suppress airway hyperresponsiveness, eosinophilia, and mucus hypersecretion in the asthmatic mouse model showing an efficacy similar to that of dexamethasone one (Lee et al., 2009). On the other hand experiments with OVA sensitized guinea pigs showed polyphenolic compounds to be more effective than their separate components, such as resveratrol, in the anti-inflammatory effects in the airways (Joskova et al., 2013). Mast cells play a key role in the pathogenesis of asthma and a resveratrol glucoside, polydatin (PD), was shown to suppress mast cell degranulation upon cross-linking of the high-affinity IgE receptors (FceRI), significantly decreasing FcεRI-mediated Ca²⁺ increase, in a mouse model of mast cell-dependent passive cutaneous anaphylaxis (Yuan et al., 2012). Respiratory syncytial virus (RSV) is the most important cause of severe infections of lower respiratory tract in infants and airway inflammation and AHR consequent to RSV infections have been associated with chronic wheezing and asthma during childhood. Resveratrol-mediated gamma interferon reduction was shown to prevent airway inflammation and AHR in RSV-infected immunocompromised mice (Zang et al., 2011).

**COPD**

Chronic obstructive pulmonary disease (COPD) is another chronic inflammatory airway disease involving poorly reversible airflow limitation, an increase in mucus producing cells and destruction of the lung parenchyma (emphysema) (Hogg and Timens, 2009). COPD is characterized by an abnormal persistent inflammatory response to cigarette smoke (CS) and noxious environmental stimuli. CS is known to cause oxidative stress and deplete glutathione (GSH) levels in alveolar epithelial cells. Resveratrol was shown to attenuate CS-mediated GSH depletion by inducing GSH synthesis and to protect epithelial cells by reversing CS-induced posttranslational modifications of nuclear factor erythroid 2-related factor 2 (Nrf2, an antioxidant-activated transcription factor that recently emerged as a critical regulator of cellular defense against oxidative and inflammatory lesions) (Kode et al., 2008). A very recent paper showed that resveratrol attenuates the release of inflammatory cytokines from human bronchial smooth muscle cells exposed to lipoteichoic acid, a major pathogen-associated molecular pattern of gram-positive bacteria increasing corticosteroid-resistant airway inflammation in COPD (Knobloch et al., 2014), and the authors suggested resveratrol as an alternative anti-inflammatory therapy in infection-induced COPD exacerbations showing that resveratrol is superior to corticosteroid dexamethasone in suppressing the release
of those inflammatory cytokines/chemokine. The same research group previously showed that resveratrol impairs the release of steroid-resistant cytokines from bacterial endotoxin-exposed alveolar macrophages (Knobloch et al., 2011) and smooth muscle cells (Knobloch, et al., 2010). Moreover it has been shown that resveratrol inhibits inflammatory IL-8 and GM-CSF cytokine release from alveolar macrophages in COPD (Culpitt et al., 2003).

In a recent study showing epigenetic regulation of DNA damage and senescence as pathogenetic mechanisms linked to endothelial progenitor dysfunction in COPD patients it was demonstrated that resveratrol treatment rescued the senescent phenotype of blood outgrowth endothelial cells from COPD patients (Paschalaki et al., 2013).

Moreover, since enhanced autophagy (a cellular process that eliminates long-lived proteins and damaged organelles through lysosomal degradation pathway) may be involved in the pathogenesis of COPD as it occurs in the lungs of patients with COPD, as well as of mice experimentally exposed to CS (Ryter and Choi, 2010). It is noteworthy that resveratrol was shown to attenuate CS-induced autophagy (Hwang et al., 2010) and the resveratrol derivative Vam3 was demonstrated to attenuate CS-induced autophagy (Shi et al., 2012b).

In an established animal model of COPD resveratrol inhalation was shown to alleviate rat COPD lung injury accompanied by amelioration of pathological changes (Zhou et al., 2008). In the same rat model resveratrol has been very recently shown to alleviate endoplasmic reticulum stress and attenuate alveolar epithelial apoptosis (Li et al., 2014).

The potentially wide role of resveratrol in treatment of COPD has been previously considered by several papers (Kamholz, 2006; Rahman, 2006; Rahman and Kilty, 2006; Sharafkhaneh et al., 2007; Wood et al., 2010; Ito et al., 2012; Shi et al., 2012a,b; Siedlinski et al., 2012; Biswas et al., 2013).

**Fibrosis**

Fibrosis of parenchymal organs is caused by prolonged injury, inflammatory diseases, deregulation of the normal processes of wound healing, and extensive deposition of extracellular matrix proteins. Idiopathic pulmonary fibrosis (IPF) is a progressive and eventually fatal parenchymal lung disease of unknown etiology with no current cure but very few pharmacologic treatment options which only slow down disease progress, including the anti-oxidant N-acetylcysteine (NAC) (Behr et al., 2009; Rafii et al., 2013). Resveratrol has been shown to produce a striking antifibrotic effect in rodent models of: i) renal (Li et al., 2010; Huang et al., 2014; Liang et al., 2014), ii) cardiac (Olson et al., 2005; Aubin et al., 2008; Sutra et al., 2008; Gupta et al., 2013).

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**Fig. 1.** Effects of resveratrol on α-SMA deposition in ex-vivo human lung fibroblasts. Human lung fibroblasts were conventionally cultured, starved for 24h and afterwards treated or not with resveratrol (10 μM⁻¹) 30 min before stimulation with TGF-β (10 ng/ml) for 48 h in serum free medium. Fixed cells were incubated overnight with primary antibody anti α-SMA, next incubated with secondary antibody Goat F(ab)'. Fragment Anti-Mouse IgG (H+L)-FITC and DAPI. Digital images were taken using fluorescence microscope coupled with digital camera (x20 magnification). In the upper panel are shown representative pictures obtained with FITC filter and in the lower one DAPI images were superimposed to show nuclei.
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2014) and iii) hepatic fibrosis (Chavez et al., 2008; Lee et al., 2010; Hong et al., 2010). In the lung context, a resveratrol protective role was demonstrated in bleomycin-induced pulmonary fibrosis in rats, attenuating oxidative injury and fibrosis due to bleomycin by its antioxidant properties (Sener et al., 2007). Importantly, a promising therapeutic potential of resveratrol was recently shown in the same bleomycin-induced pulmonary fibrosis model (Akgedik et al., 2012). In a human in vitro model we provided evidence that resveratrol inhibits both fibroblast proliferation and differentiation into myofibroblast, two critical stages of the lung fibroblast activation that play a crucial role in the fibrotic process (Fagone et al., 2011). By using immunocytochemistry we show in Figure 1 the resveratrol inhibitory effect on TGF-β-induced intracellular deposition of α-SMA fibers, the hallmark of myofibroblast differentiation.

In another in vitro human model of fibrogenic response to paraquat (PQ) exposure (a fibrosis inducing herbicide) it was shown that resveratrol attenuates PQ-induced reactive oxygen species production, inflammation, and fibrotic reactions by activating Nrf2 signaling in mouse embryonic fibroblasts (He et al., 2012). Very recently resveratrol has been shown to ameliorate LPS-induced epithelial-mesenchymal transition (EMT) and pulmonary fibrosis through suppression of oxidative stress and TGF-β1/Smad signaling pathway (Zhang et al., 2014b).

Conclusions

Despite the large number of promising preclinical study results, both in vitro and in vivo, none of the 88 studies on resveratrol nowadays registered on https://clinicaltrials.gov regards lung diseases. We hope that in the near future resveratrol putative therapeutic effects may be studied, especially in COPD and IPF settings.

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