Mechanisms of Ageing and Development xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

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

Original article

Suppressive effects of sirtinol on human cytomegalovirus (hCMV) infection and hCMV-induced activation of molecular mechanisms of senescence and production of reactive oxygen species

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ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form
28 December 2015
Accepted 30 December 2015
Available online xxx

Keywords:
Sirtinol
hCMV infection
hCMV-induced cellular senescence
Reactive oxygen species

ABSTRACT

Substantial evidence suggests that chronic human cytomegalovirus (hCMV) infection contributes significantly to T-cell immunosenescence and adverse health outcomes in older adults. As such, it is important to search for compounds with anti-hCMV properties. Studies have shown that resveratrol, a sirtuin activator, suppresses hCMV infection. Here we report suppressive effects of sirtinol, a sirtuin antagonist, on hCMV infection and its cellular and molecular consequences. Human diploid fibroblast WI-38 cells were infected by hCMV Towne strain in the absence or presence of sirtinol. hCMV replication was measured using qPCR. Senescent phenotype was determined by senescence-associated β galactosidase (SA-β-Gal) activity. Expression of hCMV immediate early (IE) and early (E) proteins and senescence-associated proteins (pRb and Rb, $p16^{INK4}$, and p53) and production of reactive oxygen species (ROS) were assessed using standard laboratory assays. The results demonstrated that sirtinol suppressed hCMV infection as well as hCMV-induced activation of molecular mechanisms of senescence and ROS production. While underlying molecular mechanisms remain to be elucidated, these findings indicate sirtinol as a novel and potent anti-hCMV agent with the potential to be developed as an effective treatment for chronic hCMV infection and its cellular and molecular consequences that are important to ageing and health of older adults.

1. Introduction

Human cytomegalovirus (hCMV) is a large DNA virus that can persist in cells of the myeloid lineage, such as monocytes and macrophages, establishing chronic or persistent infection in some immunocompetent individuals (Smith et al., 2004; Wreghitt et al., 2003). Chronic CMV infection is highly prevalent in older adults based on anti-CMV IgG serology. Substantial evidence suggests that chronic CMV infection contributes significantly to age-related T-cell immunosenescence and adverse health outcomes (Leng et al., 2011b; Pawelec et al., 2005). For example, a large number of studies have shown clonal expansion of $CD4^+$ and $CD8^+$ T cells specific to CMV pp65 or immediate early (IE)-1 epitopes in seropositive older persons (Hadrup et al., 2006; Khan et al., 2004; Koch et al., 2007; Ouyang et al., 2003; Pourgheysari et al., 2007; Vescovini et al., 2007). Several studies including our own have demonstrated broad impact of chronic CMV infection on T-cell immunity beyond those to pp65 or IE-1 epitopes including significant alterations of commonly identified T-cell phenotypes (Di Benedetto et al., 2015; Li et al., 2014a; Olsson et al., 2000; Sylwester et al., 2005). In addition to its impact on T-cell immunity, hCMV has been reported to induce cellular senescence (Noris et al., 2002; Wolf et al., 2012). At the population level, anti-CMV seropositivity has been proposed as a key component of "immune risk profile" which predicted mortality in the Swedish OCTO and NONA immune studies (Strindhall et al., 2007; Wikby et al., 2006), and others have shown significant associations between positive anti-CMV IgG serology and frailty, disability, and mortality (Aiello et al., 2008; Roberts et al., 2010; Schmaltz et al., 2005; Wang et al., 2010). More recently, we have observed that presence of hCMV viral DNA in peripherhal blood monocytes as detected by a nested PCR-based assay is likely a

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http://dx.doi.org/10.1016/j.mad.2015.12.005
0047-6374/

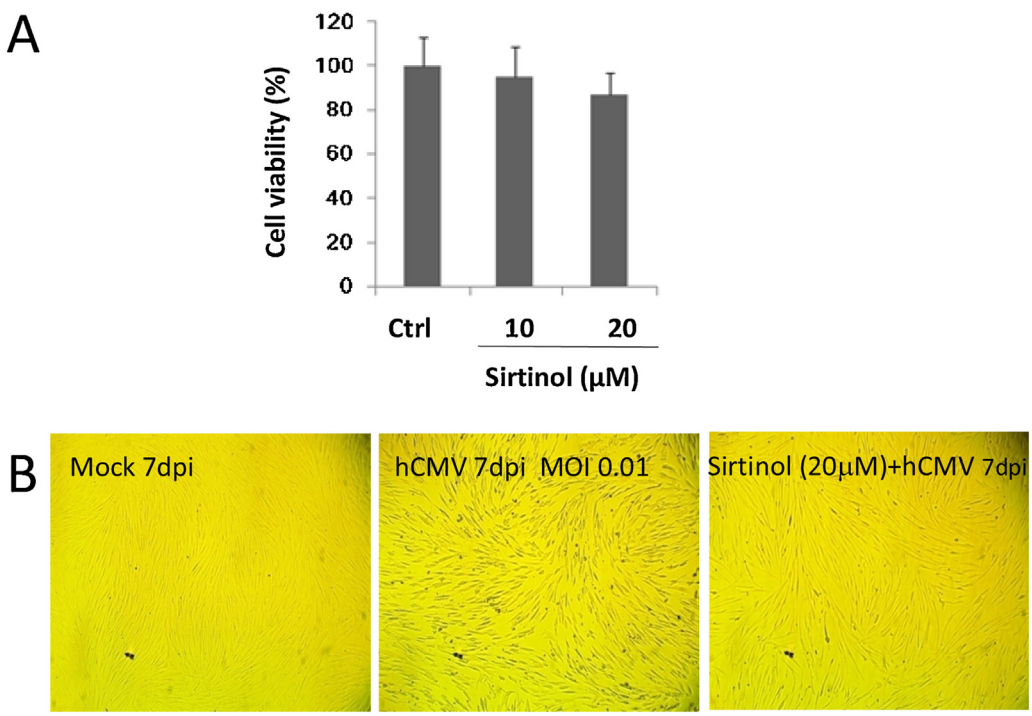

Fig. 1. Effect of sirtinol treatment on cell viability of low passage WI-38 cells (30PD) (panel A) and effects of hCMV infection alone or hCMV infection with pretreatment of sirtinol on morphological changes of low passage WI-38 cells (panel B). For panel A, WI-38 cells (3×10^4) were cultured in DEME-0.2% FBS for 48-72 h and then treated with sirtinol at indicated concentration for 48 h and then with MTT for 4 h. The reaction was stopped by DMSO and cell viability was determined spectrophotometrically at 490 nm. For panel B, under the same culture condition, WI-38 cells were pretreated with sirtinol (20 μM) for 2 h followed by hCMV inoculation at MOI of 0.01 and cultured for additional 7 days. Representative photographs shown are from 4 repeated experiments taken at day 7 post infection (dpi) using a digital camera with experimental conditions stated on each photograph.

better indicator of chronic CMV infection than positive anti-CMV IgG serology in terms of its associations with pp65-specific $CD8^+$ T-cell expansion and immune activation in older adults (Leng et al., 2011a,b), and our longitudinal study, albert at only two time points with a small sample size, has confirmed this obersavation and linked chronic CMV infection to elevated inflammation (Li et al., 2014b). Because of the significant adverse health impact of this viral infection, searching for effective interventional strategies has benn identified by the Institute of Medicine as a top priority (Arvin et al., 2004; Stratton and Lawrence, 2000).

Sirtuins (SIRT), particularly SIRT-1, are key regulators of metabolism that promote cell survival and extend lifespan (Cohen et al., 2004). Over the years, a number of sirtuin activators and inhibitors have been identified and evaluated for their biological activities (Villalba and Alcain, 2012). Resveratrol, probably the most extensively studied SIRT-1 activator, is known to promote mitochondrial biogenesis, suppress apoptosis, and reduce signs of aging (Smith et al., 2009; Pearson et al., 2008). Resveratrol was also reported to have suppressive effect against hCMV infection (Evers et al., 2004). Sirtinol and its analogues are sirtuin antagonists targeting to SIRT-1 and SIRT-2 (Grozinger et al., 2001; Mai et al., 2005). At a high concentration (50 μM or higher), sirtinol has been shown to induce senescence-like growth arrest in human breast cancer MCF-7 and lung cancer H1299 cells (Ota et al., 2006; Li et al., 2008). Blockage of SIRT-1 activity by sirtinol and other inhibitors led to endothelial dysfunction in the rat aorta (Zarzuelo et al., 2013). In our study of hCMV infection in human diploid fibroblasts, sirtinol was employed to block SIRT-1 activity in an attempt to antagonize suppressiion of reveratrol on hCMV infection. To our supprise, sirtinol inhibited hCMV infection as well. Here, we report such supprising effect of sirtinol. We also describe the suppressive effect of sirtinol on hCMV-induced activation of molecular mechanisms of senescence and production of reactive oxygen species (ROS), likely secondary to its supprssion of hCMV infection.

2. Materials and methods

2.1. Sirtinol and antibodies

Sirtinol (2-[(2-hydroxynaphthalen-1-ylmethylene)-amino]-*N*-(1-phenyl-ethyl)-benzamide) was purchased from Sigma–Aldrich (St. Louis, MO) and prepared in 20 mM stocks in dimethylsulfoxide (DMSO) stored at −20 °C until use.

Primary antibodies including anti-p53, anti-$p16^{INK4}$, and anti-β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-retinoblastoma (Rb) and anti- phosphorylated Rb (pRb^{Ser780}) were purchased from Cell Signaling Technology (Danvers, MA). Anti-hCMV immediate-early (IE) proteins at apparent molecular weights of 86, 68–72, and 38 kD primary antibody was obtained from Millipore Corporation (Billerica, MA) and Anti-hCMV early protein (UL44) was from Virusys Corporation (Randallstown, MD).

2.2. Cell culture and hCMV infection

Primary human embryonic lung diploid fibroblasts (WI-38) were purchased from the American Tissue Culture Collection (ATCC, Manassas, VA) and were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were young at population doubling (PD) of 32 or less and became replicative senescent at PD50 or higher. The cultured cells were split in ratios of 1:2 or 1:4 when the confluence of the culture was over 80%. The cumulative population doublings were calculated as $\log_2 (D/D_0)$, where D and D_0 are defined as the density of cells at the time of harvesting and seeding, respectively. All experiments were performed using cells that were between 27–32 PD for hCMV infections unless indicated otherwise.

Please cite this article in press as: Mao, G., et al., Suppressive effects of sirtinol on human cytomegalovirus (hCMV) infection and hCMV-induced activation of molecular mechanisms of senescence and production of reactive oxygen species. Mech. Ageing Dev. (2016), http://dx.doi.org/10.1016/j.mad.2015.12.005