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Aspirin attenuates vinorelbine-induced endothelial inflammation via modulating SIRT1/AMPK axis

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ABSTRACT

Vinorelbine (VNR), a semisynthetic vinca alkaloid acquired from vinblastine, is frequently used as the candidate for intervention of solid tumors. Nevertheless, VNR-caused endothelial injuries may lead a mitigative effect of clinical treatment efficiency. A growing body of evidence reveals that aspirin is a potent antioxidant and anti-inflammation drug. We investigated whether aspirin attenuate VNR-induced endothelial dysfunction. Human endothelial cells (EA.hy 926) were treated with VNR to cause endothelial inflammation. Western blotting, ROS assay, ELISA were used to confirm the anti-inflammatory effect of aspirin. We confirmed that VNR supresses SIRT1 expression, reduced LKB1 and AMPK phosphorylation as well as enriched PKC activation in treated endothelial cells. Furthermore, the membrane translocation assay displayed that the levels of NADPH oxidase subunits p47phox and Rac-1 in membrane fractions of endothelial cells were higher in cells that had been treated with VNR for than in untreated cells. We corroborated that treatment of Aspirin significantly diminishes VNR-repressed SIRT1, LKB1 and AMPK phosphorylation and VNR-promoted NADPH oxidase activation, however, those findings were vanished by SIRT1 and AMPK siRNAs. Our data also shown that Aspirin represses VNR-activated TGF-beta-activated kinase-1 (TAK1) activation, inhibited the interaction of TAK1/TAK-binding protein1 (TAB1), suppressed NF-kappa B activation and pro-inflammatory cytokine secretion. We demonstrated a novel connection between VNR-caused oxidative damages and endothelial dysfunction, and provide further insight into the protective effects of aspirin in VNR-caused endothelial dysfunction.

1. Introduction

Vinca alkaloids are considered as the most efficacious classes of clinical anticancer drugs. Vinorelbine (VNR), a semisynthetic vinca alkaloid acquired from vinblastine, is frequent used as the candidate for intervention of solid tumors, such as non-small cell lung cancer or breast cancer [1]. However, many side effects of VNR were reported in whilom studies, for example, venous irritation, vascular pain, phlebitis, and necrotizing vasculitis. Those side effects were approved for restricting clinical treatment efficiency [2].Indeed, VNR-caused endothelial inflammation plays a provide role in suppressing efficiency of chemotherapy. VNR facilitates both

Abbreviations: VNR, vinorelbine; SIRT1, sirtuin 1; AMPK, AMP-activated protein kinase; PKC, protein kinase C; LKB1, protein liver kinase B1; TAK1, TGF-β-activated kinase-1; TAB1, TAK-binding protein-1; ROS, reactive oxygen species; DCF-AM, 2′,7′-dichlorofluorescein acetoxymethyl ester; p38 MAPK, p38 mitogen-activated protein kinase; DPI, diphenyleneiodonium; IL-6, interleukin-6.

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apoptosis and inflammation in endothelial cells, Yamada et al. revealed VNR mediates endothelial cell apoptosis via facilitating reactive oxygen species (ROS) production and suppressing antioxidant enzyme activity [3]. Tsai et al. also reported the VNR mediates oxidative damage of human endothelial cells by inhibiting of AMPK, increasing of PKC and NADPH oxidase activation, decreasing of AKT/eNOS expression as well as increasing of NF-κB-mediated pro-inflammatory responses [4]. SIRT1 (Sirtuin 1) is recognized to act an important role in regulating cellular physiological processes, such as metabolism, cells degeneration, cell growth and cells survival. In human endothelial cells, SIRT1 regulates endothelial cells anti-aging as well as protects against endothelial inflammation [5,6]. Some survival genes or stress-resistance related genes are targeted of SIRT1, such as, mTOR, PI-3K, PPAR-γ or p53 [7]. SIRT1 is also shown to enhance antioxidant enzymes activity and inhibit free radical-mediated oxidative injuries via decreasing NADPH oxidase activation [8,9]. Furthermore, previous study reported the expression level and activity of SIRT1 were reduced in inflammatory endothelial cells [10].

AMP-activated protein kinase (AMPK) is a serine/threonine protein kinase and it is uniquely expressed in mammalian cells and involved in the regulation of energy balance and cellular metabolism. AMPK also modulate a number of signaling cascades that are expected to have anti endothelial cells dysfunction, such as the attenuation of free radical formation [11]. Moreover, AMPK is phosphorylated by tumor suppressor kinase liver kinase B1 (LKB1) [12], whilom study reported that LKB1/AMPK pathway is attenuated in endothelial cells under oxidative stress, this finding can appear from the impairment of SIRT1 expression, which causes to facilitate further pro-inflammatory and pro-apoptotic responses [13].

Aspirin (acetylsalicylic acid) is famous of the antiplatelet effects and anti-inflammation effects, for example, Aspirin is shown to functional attenuate acute myocardial infarction (MI) by inhibiting of cyclooxygenase enzymes [14–16]. Aspirin has also been shown to well described about its cytoprotective capabilities such as enhancing endothelium-dependent arterial relaxation via activating the release of nitric oxide from the vascular endothelium [17]. Aspirin has been validated as a regulator to mitigate PKC activation, an upstream regulator of NADPH oxidase activation and eNOS dysfunction, and that chronic administration of Aspirin causes to a repressive effect in NADPH oxidase activity [18,19]. Ou et al. first time demonstrated that pretreatment of Aspirin protects against resistin-facilitated endothelial cells inflammation as well as promote cells survival via involving AMPK and PI-3K/AKT mechanism [20]. Although the anti-inflammation and antioxidant ability of Aspirin was well investigated, so far, whether Aspirin could protect human endothelial cells inflammation from VNR-provided injuries are still largely unknown. Thus, in this present study, we pursued to explore whether Aspirin could protect against VNR- mediated endothelial oxidative injuries through the modulation of SIRT 1, and if so, whether AMPK, a negative regulator of both PKC-activated NADPH oxidase and pro-inflammatory signaling was involved in the process.

2. Materials and methods

2.1. Reagents

Fetal bovine serum, DMEM and trypsin-EDTA were obtained from Gibco (Grand Island, NY, USA); Vinorelbine, Aspirin, ethylene

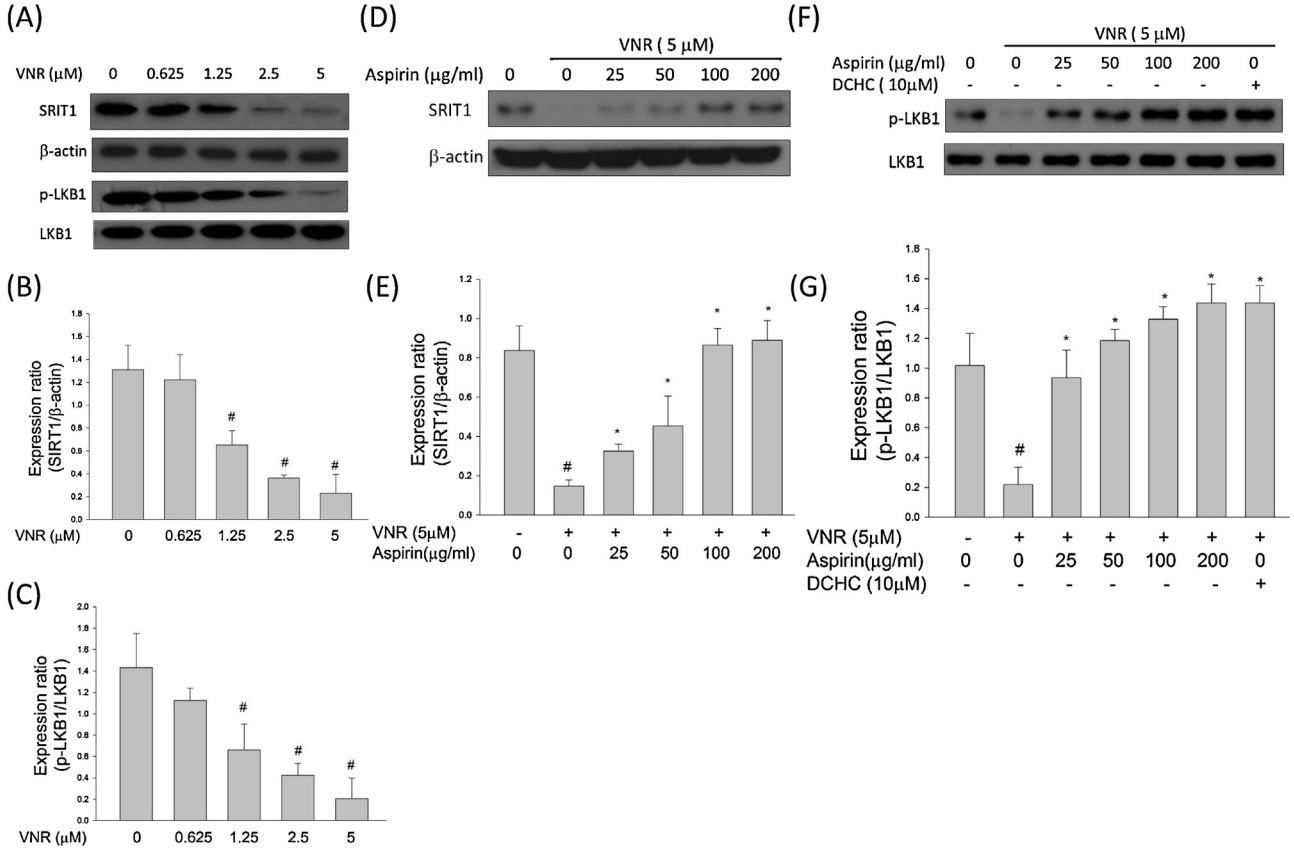

Fig. 1. VNR causes dysfunction of SIRT1 and LKB1 in EA.hy 926 cells and the inhibitory effect of Aspirin on VNR-impaired endothelial SIRT1 and LKB1 activation. EA.hy 926 cells stimulated with VNR (0.625–5 μM) 24 h to examine the SIRT1 and LKB1 expression level ((A)–(C)). EA.hy 926 cells pretreated with Aspirin (25–200 μg/ml) for 2 h, followed by exposure to VNR for a further 24 h. At the end of the incubation period, level of both SIRT1 and phosphorylated LKB1 were determined by immunoblotting. The protein levels of SIRT1 were normalized to the level of β-actin ((D) and (E)), p-LKB1 were normalized to the level of LKB1 ((F) and (G)). Data are means SE of 3 different experiments. $^{\#}$ $P < 0.05$ compared with untreated control EA.hy 926 cells. * $P < 0.05$ compared with VNR-stimulated EA.hy 926 cells.