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Original Research

Varenicline promotes endothelial cell migration by lowering vascular endothelial-cadherin levels via the activated α7 nicotinic acetylcholine receptor–mitogen activated protein kinase axis

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ABSTRACT

Varenicline is a widely used and effective drug for smoking cessation. Despite its efficacy, varenicline increases the risk of cardiovascular disease. We previously demonstrated that varenicline aggravates atherosclerotic plaque formation in apolipoprotein E knockout mice. However, little is known about its effects in vascular endothelial cells. Therefore, we examined whether varenicline promotes migration of human umbilical vein endothelial cells (HUVECs) using the Boyden chamber assay. Varenicline (100 μM) markedly promoted migration of HUVECs and decreased expression of vascular endothelial (VE)-cadherin, an endothelial adhesion molecule. Extracellular signal-regulated kinase (ERK), p38 and c-Jun N-terminal kinase (JNK) signaling were markedly activated by varenicline. Methyllycaconitine (MLA; 100 nM), an α7 nicotinic acetylcholine receptor (nAChR) antagonist, but not dihydro-β-erythroidine hydrobromide (DHβE; 20 μM) blocked varenicline-stimulated migration and varenicline-activated ERK, p38 and JNK signaling in HUVECs. MLA (100 nM), PD98059 (an ERK inhibitor; 20 μM), SB203580 (a p38 inhibitor; 20 μM) and SP600125 (a JNK inhibitor; 20 μM) also blocked cell migration and varenicline-induced downregulation of VE-cadherin expression in HUVECs. These findings suggest that varenicline promotes HUVEC migration by lowering VE-cadherin expression due to activated ERK/p38/JNK signaling through α7 nAChR. These processes probably contribute to varenicline-aggravated atherosclerotic plaque. Hence, an increased risk of cardiovascular events upon varenicline treatment might occur and must be considered in patients with cardiovascular diseases.

1. Introduction

It has been well demonstrated that smoking is a major risk factor for the induction and development of cardiovascular disease, ischemic heart disease, chronic obstructive pulmonary disease and cancer (Boyle, 1997). One of the main causes of cardiovascular disease, atherosclerosis is characterized by endothelial cell dysfunction, lipid deposition and inflammation. Endothelial cell migration is a critical angiogenic process within atherosclerotic plaque (Heeschen et al., 2001; Ross 1999), which contributes to initiation, development and rupture of atherosclerotic plaques. Specifically, angiogenesis associated with endothelial cell migration strongly induces and/or promotes infiltration of inflammatory cells, lipid deposition, intraplaque hemorrhage and hemosiderin deposits (Folkman 1995; Fosbrink et al., 2006; Groszen and Grundy, 1980; Kockx et al., 2003; Michel et al., 2011; Moreno et al., 2006). Downregulation of vascular endothelial (VE)-cadherin occurs prior to endothelial cell migration in the early stage of angiogenesis; this event is induced through activation of extracellular signal-regulated kinase (ERK) (Wu et al., 2003). Liu et al. demonstrated that human umbilical vein endothelial cell (HUVEC) migration was enhanced by ERK pathway activation (Liu et al., 2015). Furthermore, activation of p38 mitogen activated protein kinase (MAPK) via vascular endothelial growth factor and urokinase plasminogen activator mediates cell migration in human endothelial cells (Rousseau et al., 1997; Yu et al., 2004). The c-Jun N-terminal kinase (JNK) signaling pathway is required for basic fibroblast growth factor-mediated downregulation of VE-cadherin in HUVECs (Wu et al., 2008). It is therefore conceivable that sequential MAPK activation–VE-cadherin downregulation–endothelial cell migration contributes substantially to atherosclerosis formation.

Abbreviations: nAChR, nicotinic acetylcholine receptor; HUVECs, human umbilical vein endothelial cells; VE-cadherin, vascular endothelial-cadherin; MLA, methyllycaconitine; DHβE, dihydro-β-erythroidine hydrobromide; ERK, extracellular signal-regulated kinase; MAPK, mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-kappa B

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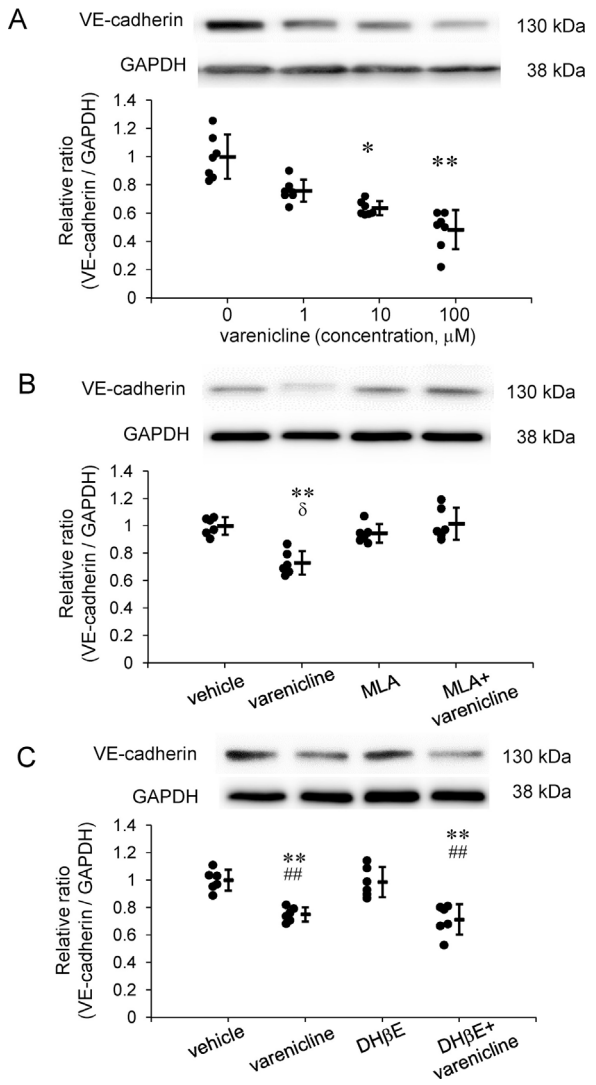

Fig. 1. Varenicline decreases the protein levels of VE-cadherin in HUVECs. Representative immunoblots (upper panel) and quantitative analyses of band intensities (bottom panel) showing protein expression of VE-cadherin in (A), (B) and (C). (A) Cells were treated with various concentrations of varenicline (1, 10 and 100 μM) for 24 h. GAPDH was used as a loading control. Each dot represents data obtained from individual experiments. The means ± SD of seven separate experiments (n = 3 wells each) are shown. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with 0 μM. (B) and (C) Cells were treated with vehicle or varenicline (100 μM) for 24 h in the absence or presence of the α7 nAChR antagonist MLA (100 nM) (B) or α4β2 nAChR antagonist DHβE (20 μM) (C). MLA and DHβE were added to cells 30 min before vehicle or varenicline treatment. GAPDH was used as a loading control. Each dot represents data obtained from individual experiments. The means ± SD of five or six separate experiments (n = 3 wells each) are shown. $^{**}P < 0.01$ compared with vehicle. $^{\delta}P < 0.05$ compared with MLA + varenicline. $^{\#\#}P < 0.01$ compared with DHβE.

Varenicline is a selective α4β2 nicotinic acetylcholine receptor (nAChR) partial agonist and an α7 nAChR full agonist (Coe et al., 2005; Mihalak et al., 2006). It has been established that the pharmacological action of varenicline occurs mainly through α4β2 nAChR, and that treatment with varenicline is safer and more effective than nicotine replacement therapy for smoking cessation (Gonzales et al., 2006; Jorenby et al., 2006; Nakamura et al., 2007). However, varenicline shows adverse effects including headache, nausea, abnormal dreams and insomnia (Hays et al., 2008; Oncken et al., 2006). In addition, the risk of cardiovascular events has been reported to increase in patients receiving varenicline (Hays et al., 2008; Singh et al., 2011). We previously reported that varenicline aggravates the formation of atherosclerotic plaques through α7 nAChR in apolipoprotein E knockout mice

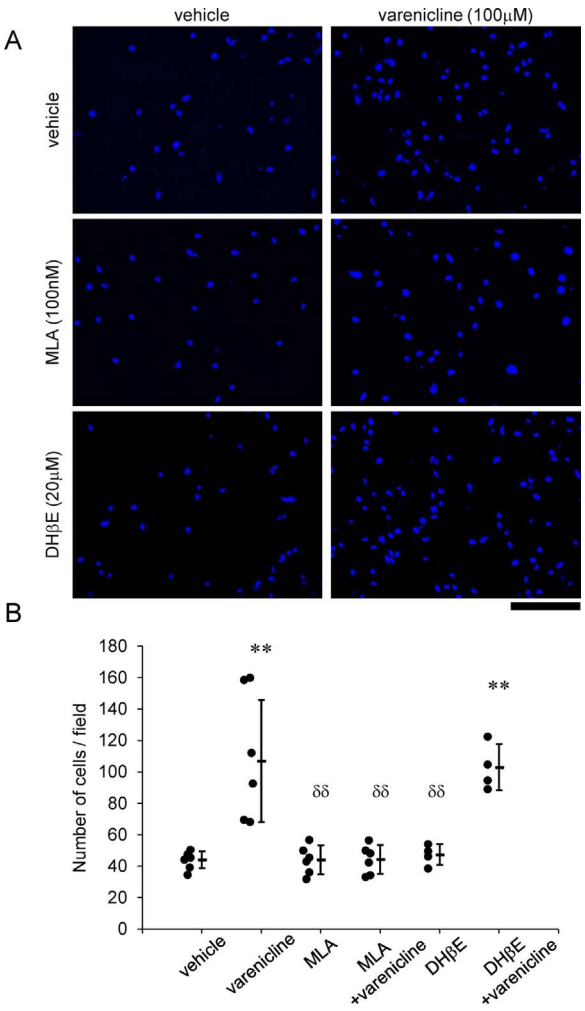

Fig. 2. Varenicline promotes HUVEC migration. (A) Representative photographs showing DAPI-stained migrating cells. Cells were exposed to vehicle or varenicline (100 μM) in the absence or presence of MLA (100 nM) or DHβE (20 μM). MLA and DHβE were added to cells 30 min before vehicle or varenicline treatment. Migration assays were carried out with Boyden chambers for 4 h after the addition of varenicline. Scale bar = 100 μm. (B) Dot graphs showing the number of migrating cells per field (0.1 mm^2/field). Each dot represents individual data (n = 6 experiments in triplicate). Data are the means ± SD of six separate experiments. $^{**}P < 0.01$ compared with the vehicle. $^{\delta\delta}P < 0.01$ compared with varenicline.

and that varenicline stimulates α7 nAChR to enhance oxidized low-density lipoprotein (LDL) uptake by increasing expression of scavenger receptors through activation of ERK signaling (Koga et al., 2014; Kanaoka et al., 2017). However, its mechanism of action was not determined. We hypothesized that varenicline lowers VE-cadherin expression through the activated α7 nAChR–MAPK signaling pathway to promote endothelial cell migration associated with angiogenesis, leading to atherosclerotic aggravation. In the present study, we elucidated whether varenicline promotes migration of HUVECs and clarified its mechanism of action *in vitro*.

2. Methods and materials

2.1. Drugs

Varenicline tartrate (7,8,9,10-tetrahydro-6,10-methano-6H-pyrazino [2,3-h][3] benzazepine,(2R,3R)-2,3-dihydroxybutanedioate) and methyllycaconitine ([1α,4(*S*),6β,14α,16β]-20-ethyl-1,6,14,16-tetramethoxy-4-[[[2-(3-methyl-2,5-dioxo-1-pyrrolidinyl)benzoyl]oxy]methyl]aconitane-7,8-diol citrate [MLA, an α7 nAChR antagonist]) were purchased from