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The effects of nicotine on vascular smooth muscle cell chemotaxis induced by thrombospondin-1 and fibronectin

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Abstract

BACKGROUND: Vascular smooth muscle cell (VSMC) migration is an important process in many vascular disorders. Nicotine, thrombospondin-1 (TSP-1) and fibronectin (Fn) separately induce VSMC migration. The hypothesis of this study was that nicotine treatment of vascular cells would augment TSP-1-induced and Fn-induced VSMC migration.

METHODS: VSMCs or endothelial cells (ECs) were treated with serum-free medium or nicotine. Migration of VSMCs was assessed using a modified Boyden chemotaxis chamber to serum-free medium, TSP-1, Fn, EC basal medium, and conditioned EC medium or nicotine-treated conditioned EC medium alone or with supplemented TSP-1 or Fn.

RESULTS: Nicotine treatment increased VSMC chemotaxis to serum-free medium, but TSP-1 or Fn had no further effect on chemotaxis. Conditioned EC and nicotine-treated conditioned EC enhanced VSMC chemotaxis, which was further augmented by Fn supplementation.

CONCLUSIONS: Nicotine-stimulated EC derived factors induce VSMC migration, which is augmented by the addition of Fn.

Vascular smooth muscle cell (VSMC) migration is a process that occurs in the development of several vascular pathologies, including intimal hyperplasia and atherogenesis. Cigarette smoking is a known risk factor for cardiovascular diseases.[1,2] Nicotine, a component of cigarette smoke, stimulates VSMC migration, as well as exerting effects on vascular endothelial cells (ECs).[3–5]

Thrombospondin-1 (TSP-1), an extracellular matrix glycoprotein, is expressed at areas of vascular injury.[6] TSP-1 has many functions, which include stimulating VSMC migration.[6–8] TSP-1 also influences EC function by inhibiting EC proliferation and inducing EC apoptosis.[9]

Fibronectin (Fn) is a structural extracellular matrix protein that binds integrins and other extracellular matrix components. Furthermore, plasma Fn acts as an acute phase reactant under conditions of stress and inflammation. Fn stimulates VSMC migration through signaling pathways that differ from TSP-1.[6,8]

The purpose of this study was to examine the effects of nicotine on TSP-1-induced and Fn-induced VSMC migration. Additionally, ECs can regulate VSMC function, and nicotine has been shown to induce EC dysfunction as well as VSMC migration.[4,5] Our hypotheses were that (1) nicotine treatment of VSMCs augments migration to TSP-1 or Fn and (2) medium harvested from ECs, or nicotine-treated ECs, augments VSMC migration to TSP-1 or Fn.

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Methods

Materials

TSP-1 (Athens Research, Athens, GA), Fn (EMD Chemicals, Gilbertstown, NJ), (±)-nicotine (Sigma-Aldrich, St. Louis, MO), human aortic VSMCs, and ECs were purchased from Cell Applications (San Diego, CA).

Cell culture

VSMCs were made quiescent with serum-free medium (SFM) and were then treated for either 24 or 48 hours with 10^{-8} mol/L nicotine, which was chosen because it falls within the range of nicotine in the serum of smokers.[10] Basal medium from untreated ECs (conditioned EC medium [CEC]) or 48-hour nicotine-treated ECs (conditioned nicotine-treated EC medium [CNEC]) was harvested for the 2nd set of experiments.

Cell viability assay

Cell viability and size were assessed with a trypan blue exclusion assay using a Countess Cell Counter (Invitrogen, Carlsbad, CA).

Chemotaxis assay

A modified Boyden microchemotaxis chamber was used as previously described.[7] Experiments ran for 4 hours at 37°C (n = 3) and were repeated in triplicate. First, migration of VSMCs with or without nicotine treatment (24 or 48 hours) was assessed to SFM, TSP-1 (20 μg/ml), or Fn (20 μg/ml). Next, VSMCs with or without nicotine treatment (48 hours) were migrated to SFM, TSP-1, Fn, CEC with or without TSP-1 or Fn supplementation, or CNEC with or without TSP-1 or Fn supplementation. Cells migrated were recorded as cells per 5 high-power fields (400×) and then converted to either percentage negative or positive control.

Statistical analysis

Data are expressed as mean ± SEM. Analysis of variance with post hoc testing was used for all data analysis. Statistical significance was set at $P < .05$.

Results

Cell viability and size

VSMCs retained similar viability after treatment with SFM (95 ± 1%), 24-hour nicotine treatment (96 ± 1%, $P = .51$), or 48-hour nicotine treatment (95 ± 1%, $P = .86$). Mean viable cell size was similar between SFM treatment (21 ± 1 μm) and 24-hour nicotine treatment (23 ± 3 μm) ($P = .35$) or 48-hour nicotine treatment (22 ± 2 μm) ($P = .62$). Additionally, ECs retained similar viability (93 ± 2 vs 96 ± 1%, $P = .32$) and cell size (20 ± 1 vs 20 ± 1 μm, $P = .97$) after 48-hour treatment with nicotine.

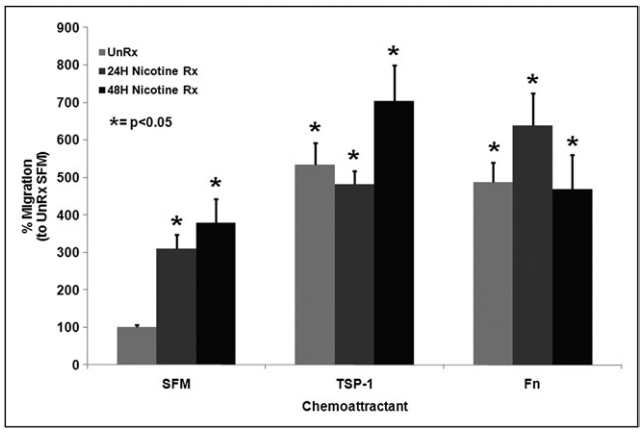

Figure 1 Effect of nicotine treatment on VSMC chemotaxis. Nicotine induced VSMC chemotaxis. TSP-1 or Fn independently induced chemotaxis but did not further augment chemotaxis in nicotine-treated VSMCs. *Difference ($P < .05$) from SFM-induced 24-hour nicotine-treated VSMC chemotaxis.

Nicotine effects on TSP-1-induced chemotaxis (comparisons with SFM negative control)

TSP-1 induced chemotaxis in VSMCs cultured in SFM (534 ± 57%; Fig. 1). Chemotaxis to SFM was increased by 24 hours (275 ± 34%) and 48 hours (380 ± 62%) of nicotine treatment. At 24 hours (482 ± 35%) or 48 hours (703 ± 96%), nicotine treatment stimulated TSP-1-induced chemotaxis. No difference existed in TSP-1-induced chemotaxis for VSMCs treated with SFM alone, 24-hour nicotine treatment ($P = .91$), or 48-hour nicotine treatment ($P = .16$).

Nicotine effects on Fn-induced chemotaxis (comparisons with negative control)

Fn stimulated chemotaxis in VSMCs cultured in SFM (486 ± 53%). Furthermore, Fn stimulated VSMC chemotaxis after 24-hour treatment with nicotine (638 ± 86%) or 48-hour nicotine treatment (468 ± 92%) (Fig. 1). No difference in chemotaxis existed between SFM-treated VSMCs and 24-hour nicotine-treated VSMCs ($P = .84$) or 48-hour nicotine-treated VSMCs ($P > .99$) when migrated to Fn.

CEC-induced and CNEC-induced chemotaxis

EC basal medium without exposure to ECs was tested as a chemoattractant, and the results were no different from SFM (not shown). Both CEC and CNEC increased VSMC chemotaxis over SFM (1,041 ± 43% and 1,241 ± 119%,