Nicotine Administration Significantly Alters Peripheral, Bone Marrow and Splenic Stem Cell Populations in C57Bl6 Mice

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Background: Smoking is known to increase circulatory mononuclear cell counts. Nicotine is a major component of cigarette smoke. Recently, it has been reported that nicotine promotes tumor growth, plaque neovascularization and wound healing through its intrinsic angiogenic properties. We investigated the potential connections between nicotine’s angiogenic and its hematopoietic or lymphopoietic properties.

Hypothesis: That nicotine can promote angiogenesis partly through its capacity to elicit the proliferation or mobilization of peripheral cells via the activation, proliferation and migration of hematopoietic stem cells (HSC) and endothelial precursor cells (EPCs).

Methods: C57Bl6 mice (n=10) were fed normal mouse chow plus 100ug/ml free base nicotine 0.4% saccharine. Control mice (n=10) were given normal chow and 0.4% saccharine ad libitum. At 2 weeks (n=5) and at 6 weeks (n=5), the animals were sacrificed and their peripheral blood, bone marrow and spleen harvested. Total cell counts for peripheral cells, bone marrow and spleen as well as splenic weight were recorded. Isolated bone marrow, splenocytes and pooled mononuclear blood cells were assessed for HSC and EPC population via FACS analysis. Statistical analyses based upon Student’s t-test.

Results: Nicotine treated animals had significantly increased mass of spleen (p<0.01) and an elevated white blood cell count (p<0.02) after six weeks. Total cell numbers of bone marrow and spleen were also significantly increased (p<0.02) in the nicotine fed mice. No significant change in body mass was evident. FACS analysis of the bone marrow and spleen showed a small augmentation (p<0.04) of splenic CD34 levels at 2 and 6 weeks of ad libitum nicotine administration. Otherwise, no major changes in the cellular subpopulations of nicotine-challenged bone marrow or spleen were observed. Nicotine did significantly increase (p<0.03) HSC and EPC levels in nicotine treated tissue.

Conclusions: Nicotine’s capability to increase bone marrow HSC and EPC levels may serve as a functional link for both nicotine’s angiogenic properties and its putative role in augmenting circulatory mononuclear cell content.

Heterologous Transplantation of CD34+ Cells From Patients With Coronary Ischemia Leads to Myocardial Salvage and Enhanced Functional Recovery in Murine Model of Myocardial Infarction

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Background: CD34+ cells (CDCs) contain an enriched fraction of endothelial progenitor cells. When obtained from healthy volunteers, they can induce neovascularization in models of myocardial infarction (MI). It is unknown whether coronary ischemia impairs the function of these cells. We investigated the hypothesis that administration of CDCs from patients with coronary ischemia could induce therapeutic myocardial neovascularization.

Methods: Mononuclear cells (MNCs) and CDCs were isolated from peripheral blood samples of healthy volunteers (n=5) and patients with positive stress test undergoing cardiac catheterization (n=7). After 2-week culture, CDCs from both groups differentiated into EPCs. After surgical ligation of the LAD, male Hsd:Rnu (athyric) rats received: A) 100,000 CDCs (healthy subjects, n=6); B) 100,000 CDCs (patients, n=9); C) 100,000 MNCs (patients, n=9); and D) PBS (control, n=11), by IV injection. Left ventricular (LV) fibrosis area was assessed histologically, LV function by echocardiography and pressure parameters, and capillary density by endothelial cell staining. Statistical analyses based upon Student’s t-test.

Results: Four weeks after administration of CDCs, capillary density (mean ± SEM) in ischemic myocardium was significantly greater in group B than in C and D (276±15.5 vs 176±4.3±11.3, and 88±3.5±4.4mm2; p<0.01), but less than in A (324±52.7±7mm2; p=0.05). LV fibrosis area was significantly reduced in B (17±6.2% vs C, 25±6.2%; p<0.05; and D: 29±8.1%; p<0.01), but larger than in A (11±2.2%±8±0.05). Percent difference in fractional shortening was significantly less marked in B than in C and D (26±4.3±2 vs -45±5.5, and -55±2.6%; p<0.01). Regional wall motion score was significantly better in B than in C and D (299±4±143 vs 163±9±139, and 168±108 mmHg/sec; p<0.01).

Conclusions: CD34+ cells transplanted from patients with myocardial ischemia achieve therapeutic neovascularization leading to myocardial salvage (as demonstrated by inhibition of LV fibrosis) and preservation of LV function after MI. These findings suggest the possible use of cell therapy strategies in the treatment of individuals with CAD. However, the ability of BAU to identify women with CAD has not been evaluated. This study aimed to compare the ability of BAU to detect CAD in women versus men. Methods: 209 outpatients (141 women, 68 men) who were either referred for clinically-indicated stress test imaging, or had a history of angiographically-proven CAD, underwent BAU under standard conditions. Flow-mediated vasodilatation (FMD) was defined as the percent change in brachial artery diameter during reactive hyperemia compared to baseline. CAD was defined as an abnormal endothelium scan (confirmed by prior or subsequent angiography), or history of angiographically-documented CAD. Receiver operator characteristic (ROC) curve analysis was performed to analyze sensitivity and specificity of FMD for detection of CAD in women and men. Results: Mean age was similar for women and men. CAD was defined as 0.008). The FMD cut-point which maximized sensitivity with the least effect on specificity for identification of CAD in women was an FMD=15% (95 sensitivity, 25 specificity). In contrast, the ‘optimal’ cutpoint in men was an FMD=10% (90 sensitivity, 43 specificity), consistent with prior published work. If the FMD cutpoint of >=10% defined in men were used to screen women, BAU would fail to diagnose 42% of the CAD cases in women. Conclusion: BAU can be a highly sensitive technique for detection of CAD in both men and women. However, mean FMD is significantly higher in women with CAD than men with CAD, and thus a significantly higher FMD cut-point must be used to identify women with CAD. These findings indicate that, in studies using FMD to evaluate cardiovascular risk, different standards should be applied for women and men.

Vascular Endothelin-1 Activity Correlates With Low-Density Lipoprotein Cholesterol Levels, but Not With Insulin Sensitivity in Hypercholesterolemic Patients

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Background: Patients with hypercholesterolemia have an increased vascular endothelin (ET1) activity, which is likely related to an enhanced production of ET1. In vitro studies have demonstrated that oxidized LDL may induce ET1 release in cultured human endothelial cells. However, other stimuli, such as insulin, have also been shown to promote ET1 synthesis in vitro. The exact mechanisms contributing to the increased ET1 activity of hypercholesterolemic patients (HCPs) in vivo have not been identified. Thus, we aimed to determine the contribution of different metabolic parameters to ET1 activity in resistance vessels of patients with hypercholesterolemia.

Methods: Twenty female normohyperlipidemic HCs and 26 normal controls (NCs) took part in the study. Forearm blood flow (FBF) responses to intraarterial infusion of the ET1 receptor blocker BG-123 (100 nmol/min for 60 min) were analyzed by venous occlusion plethysmography, and insulin sensitivity (Sclamp) was measured by euglycemic clamp.

Results: As previously shown, BG-123 administration did not result in significant changes in FBF from baseline in NCs (P=0.589), whereas it produced significant vasodilation in HCs (P=0.037). In HCs, the response to ET1 blockade was significantly correlated with total cholesterol (R=0.441, P=0.031), and LDL cholesterol (R=0.477, P=0.003), whereas no significant correlations were observed with basal plasma insulin, Sclamp, and body mass index (BMI) (all P>NS). No significant correlations between the response to ET1 blockade and total cholesterol, LDL-cholesterol, basal insulin, Sclamp and BMI were observed in NCs (all P>NS).

Conclusions: In hypercholesterolemic patients, vascular ET1 activity is significantly correlated with total and LDL-cholesterol plasma levels, but not with other metabolic parameters. Our findings indicate that, in this population, the enhanced ET1 vasoconstrictor tone is mainly secondary to the increased cholesterol levels, and suggest that cholesterol-lowering therapy may be effective in reducing ET1 activity in the vasculature of patients with primary hypercholesterolemia.