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Effects of varenicline and nicotine replacement therapy on arterial elasticity, endothelial glycocalyx and oxidative stress during a 3-month smoking cessation program

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ABSTRACT

Background and aims: The effects of medically-aided smoking cessation on vascular function and oxidative stress are not fully clarified.
Methods: One hundred eighty-eight current smokers were randomized to varenicline or nicotine replacement treatment (NRT) for a 3-month period. We assessed: (a) augmentation index (Aix) and pulse wave velocity (PWV); (b) perfusion boundary region (PBR) of sublingual microvasculature (range:5–25 μm), an index of the endothelial glycocalyx thickness, using Sideview, Darkfield imaging; (c) the exhaled CO; and (d) the malondialdehyde (MDA) and protein carbonyls (PC) plasma levels, as markers of oxidative stress, at baseline and after 3 and 12 months.
Results: After 3 months of treatment, CO, MDA, PC and Aix were decreased in all subjects (median CO: 25 *vs.* 6 ppm, MDA: 0.81 *vs.* 0.63 nmol/L, PC: 0.102, *vs.* 0.093 nmol/mg protein, Aix: 13% *vs.* 9%, $p < 0.05$) while PWV remained unchanged. Endothelial glycocalyx integrity showed a greater improvement in the varenicline than the NRT treatment (PBR range 5–9 μm: 1.07 ± 0.02 *vs.* 1.17 ± 0.02 μm, $p = 0.03$) in parallel with the greater CO reduction (5 *vs.* 7 ppm, $p = 0.02$). At 1-year follow-up, MDA, PC, Aix and PBR at 5–25 μm range were further improved in subjects who abstained from smoking (n = 84 out of 188), while the above markers and PWV deteriorated in relapsed smokers ($p < 0.05$).
Conclusions: A smoking cessation program using varenicline or NRT for 3 months resulted in a decrease of CO, oxidative stress, arterial stiffness and restored endothelial glycocalyx. These effects were more evident after varenicline treatment, likely because of a greater CO reduction, and were maintained after 1 year only in subjects who abstained from smoking.

1. Introduction

Cigarette smoking is a major modifiable risk factor for cardiovascular disease [1]. Increased arterial stiffness [2], endothelial dysfunction [3], and oxidative stress accentuation [3] represent significant pathophysiologic substrates of its toxic effects. Aortic pulse wave velocity (PWV) and augmentation index (Aix) are non-invasive and reproducible markers of arterial stiffness [4], with an independent predictive value for cardiovascular events [5]. The endothelial glycocalyx consists of glycoproteins and proteoglycans, and forms a layer that prevents the direct contact of blood cells to the endothelial surface in the vessels [6,7]. Glycocalyx damage is caused by inflammatory or atherogenic factors leading to enhanced sensitivity of vasculature to prothrombotic, vasoactive and atherogenic stimuli [6–13]. Based on these observations, the importance of integrity of the endothelial glycocalyx in vascular homeostasis has become evident [13]. Novel imaging techniques permit the non-invasive assessment of the endothelial glycocalyx thickness of the sublingual arterial microvessels by measurement of the perfusion boundary region (PBR) of the luminal wall of the

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microvessels with diameters ranging from 5 to 25 μm [11–13]. Furthermore, malondialdehyde (MDA) and protein carbonyls (PC) plasma concentrations are commonly used as valid biomarkers of oxidative stress [14,15].

Smoking cessation represents an essential component of both primary and secondary prevention of cardiovascular diseases [16,17]. In this respect, nicotine replacement therapy (NRT) and varenicline remain the mainstay of pharmacological smoking cessation interventions. Despite their widespread use and apparent efficacy, their effects on cardiovascular function have not been fully elucidated. It is known that nicotine may trigger several adverse catecholamine-mediated vascular responses [18]. Furthermore, studies have suggested that varenicline may be associated with a risk of adverse events, including cardiovascular complications [19].

The aim of this study was to evaluate the effects of a 3-month pharmacologically-assisted smoking cessation program on endothelial glycocalyx, arterial elasticity and oxidative stress and to compare the changes on the above factors across the two intervention arms (varenicline *vs.* NRT). Finally, we monitored the changes of the examined biomarkers after 1 year of follow-up.

2. Patients and methods

2.1. Study population

We measured the exhaled carbon monoxide (CO) concentration (parts per million-ppm, Bedfont Scientific, Maidstone, Kent UK) and the number of smoked cigarettes in consecutive current smoker subjects who attended the Attikon University Hospital smoking cessation clinic. Subjects with uncontrolled diabetes with an HbA1c greater than 7% and/or treated with medication other than metformin were not considered eligible for the study. A total of 228 current smokers who smoked more than 5 cigarettes per day and had an exhaled CO greater than 10 ppm were considered eligible for the study. Seventeen subjects with history of coronary heart disease or any other vascular disease, cardiac, hepatic, or renal failure, active neoplasia, alcohol abuse, psychiatric illness, pregnancy, breastfeeding, or cigar smokers were excluded from the study (Supplemental Fig. 1).

Out of the remaining 211 smokers, a total of 188 smokers gave informed consent (age: 50 ± 3; 45% female) and thus were finally included in the study. Ninety-four healthy non-smoker subjects (self-reported as never smoked and with exhaled CO < 5 ppm) with similar age and sex as the smokers (age: 49 ± 12; 47% female) were selected to serve as controls using a ratio of 1 control per 2 smokers.

Smokers were randomly allocated to two treatment arms: subjects in the first group were administered varenicline [n = 94, 50%; progressive titration for 3 months as follows: 0.5 mg OD (once daily) for 3 days, 0.5 mg BID (twice a day) for 4 days, and 1 mg BID for the remaining weeks], whilst subjects in the second group were administered nicotine patches and chewing gums (n = 94, 50%; progressive titration for 3 months as follows: patches of 15 mg for 30 days, 10 mg for 30 days, 5 mg for 30 days plus 6–8 chewing gums per day containing 2 mg of nicotine each throughout the 3-month treatment period). Exhaled CO concentration and the number of smoked cigarettes were measured at inclusion and after 3 and 12 months of follow-up.

Dyslipidaemia was defined as total cholesterol >200 mg/dl or the use of cholesterol-lowering agents; hypertension was defined as blood pressure >140/90 mmHg or use of anti-hypertensive drugs; diabetes mellitus was defined as fasting plasma glucose >125 mg/dl or use of antidiabetic drugs.

Any concomitant medical treatment was fully recorded and written informed consent was obtained in accordance with the Helsinki declaration. The study was approved by the Scientific and Ethics Committee of the General University Hospital Attikon.

2.2. Aortic stiffness, glycocalyx integrity, smoking status, and oxidative stress estimation

Measurement of vascular markers and blood sampling for oxidative stress markers were obtained at baseline, after 3 months into the smoking cessation program and twelve months after inclusion in the study. Vascular studies were performed by a single, blinded-to- treatment operator. Treatment with ACE or ARBs was withdrawn 48 h before the vascular studies and blood sampling; only diuretics were allowed for controlling BP.

2.3. Arterial stiffness

Carotid to femoral pulse wave velocity (PWV) was measured using a previously published methodology (Complior, Alam Medical, Vincennes, France) [4]. PWV was calculated as the distance between the carotid and femoral arterial pulse palpation site divided by the transit time between waves (m/s). Central augmentation index (Aix%) is defined as: 100 x (P2–P1)/PP, (where P2 = late backward systolic wave, P1 = early forward systolic wave, PP = central PP) and represents the pressure boost that is induced by the return of the reflected waves at the aorta [20]. The inter- and intra-observer variabilities were 6% and 5% for PWV, and 12% and 10%, for Aix, respectively.

2.4. Endothelial glycocalyx

We measured the perfused boundary region (PBR) of the sublingual arterial microvessels (with diameter range from 5 to 25 μm) using Sidestream Darkfield imaging (Microscan, Glycocheck, Microvascular Health Solutions Inc., Salt Lake City, UT, USA) that provides a direct, noninvasive, and fast method for the assessment of the endothelial glycocalyx using our previously published methodology [12]. The PBR is the cell-poor layer, which results from the phase separation between the flowing red blood cells (RBC) and plasma on the surface of the microvessel lumen. The PBR includes the most luminal part of glycocalyx that does allow cell penetration. Thus, an increased perfused boundary region (PBR) is consistent with deeper penetration of erythrocytes into glycocalyx, indicating a loss of glycocalyx barrier properties and is a marker of reduced glycocalyx thickness [11,12]. The measurement of endothelial glycocalyx thickness using Sidestream Darkfield imaging is easy to perform (duration of 3 min), is operator independent, has standardized methodology, provides measurements of sampling sample sites (>3000 vascular segments of sublingual microvasculature) and estimation of the glycocalyx integrity of the microvessels with a diameter ranging from 5 to 25 μm has a very good reproducibility [9], and thus is proposed as a valid method to assess endothelial integrity by the European Society of Cardiology Working Group on Peripheral Circulation [13]. The inter- and intra-observer variabilities of PBR measurements were 5.2% and 4.3%, respectively.

2.5. Laboratory assays

Malondialdehyde (MDA) was determined spectrophotometrically with a commercial kit (Oxford Biomedical Research, Rochester Hills, Mich, colorimetric assay for lipid peroxidation; measurement range 1–20 nmol/L) [14]. For the quantification of protein carbonyl (PC) content, we based on spectrophotometric measurement of 2,4-dinitrophenylhydrazine derivatives of protein carbonyls, as previously published [21] and results were expressed as nmol/mg protein. For MDA and PC, the intra-assay variability was 3.39% and