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Original article

Anti- and pro-oxidant factors and endothelial dysfunction in chronic cigarette smokers with coronary heart disease

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Abstract

Background: Endothelial dysfunction in cigarette smokers has been ascribed to increased oxidative damage. The aims of the present study were to compare the endothelial function of normotensive smokers with that of non-smokers and to examine its relation to some parameters representative of oxidative damage and of antioxidant capacity.
Methods: We investigated 32 chronic smokers (15–30 cigarettes daily) affected by coronary heart disease, ranging from acute myocardial infarction to instable angina pectoris, and 28 matched non-smokers without any definite risk factors. All subjects underwent assessment of nitric oxide (NO)-dependent endothelial function, measured as brachial artery vasodilatation in response to reactive ischemia, using a standardized echographic method. Plasma and urinary levels of NO were also measured in all subjects, as were urinary 15-isoprostane F_{2t}, plasma serum lipids, homocysteine (Hcy), ascorbic acid, retinol, tocopherol, and alpha- and beta-carotene (by high-performance liquid chromatography).
Results: Smokers showed a significantly lower NO-mediated vasodilatation response (3.50% vs. 6.18%, $p<0.001$) and higher levels of urinary NO metabolites and 15-isoprostane F_{2t}. They also had higher levels of Hcy ($p<0.001$); these values were significantly and inversely related to NO serum levels ($r=-0.512$, $p<0.001$). Moreover, smokers had a significant and corresponding reduction in circulating levels of ascorbic acid, tocopherol, and alpha- and beta-carotene.
Conclusions: The present study shows a clear relation between endothelial dysfunction (NO production impairment) and cigarette smoking, especially in the presence of high levels of LDL-cholesterol. It also defines some markers of both oxidative damage and antioxidant protective capacity in this condition. The monitoring of these factors may be advisable in order to assess the amount of endothelial damage.

Keywords: Antioxidants; Endothelial dysfunction; Cigarette smoking; Homocysteine; Isoprostane; Nitric oxide

1. Introduction

Cigarette smoking represents an independent risk factor for cardiovascular disease [1]. However, the relative role of nicotine [2–5] and of other factors (hypertension, hypercholesterolemia) [6,7] in inducing vascular damage, and particularly endothelial dysfunction, is not well-defined. The latter, which is widely considered to be an important hallmark of atherosclerosis, is often the final result of different complex mechanisms, involving the production of reactive oxygen species (free radicals and peroxylradicals) [8] and leading to reduced nitric oxide (NO) endothelial availability.

In the vasculature, endothelial NO (EDNO) plays a key role in maintaining the blood flow by modulating physiological vasodilatation [9–12]. Another important physiological reaction of NO is the formation of thionitrites or *S*-nitrosothiols (RSNOs) [13]. These adducts form in the

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Table 1
Dietary report in the groups studied, based on an empirical scoring system for the different classes of nutrients

Groups	Poultry	Meat	Leafy vegetables	Tomatoes and carrots	Fruit	Nuts	Milk	Dairy products
Non-smokers (n=28)	1.92±0.15	1.64±0.15	2.31±0.16	2.08±0.16	2.54±0.16	0.95±0.15	2.24±0.23	2.31±0.12
Smokers (n=32)	1.66±0.11	1.75±0.16	2.17±0.13	2.03±0.14	2.52±0.16	1.05±0.14	2.00±0.20	2.55±0.14

Values from 0 to 3, corresponding, respectively, to an absence of the class in the weekly diet to an optimal dietary intake, were assigned according to a local nutritional board.
Mean±S.E.

presence of a thiol compound and oxygen and have, in turn, an important role in reducing vascular tone and platelet aggregation [14,15]. Among the different kinds of RSNOs, the transformation of homocysteine (Hcy), a well-known independent risk factor for atherothrombosis, into *S*-nitroso-homocysteine seems relevant. This substance, unlike "native" Hcy, does not support hydrogen peroxide generation and, hence, cell toxicity, but shows, on the contrary, well-defined and important vasodilating and inhibiting properties of platelet aggregation and adhesion of leukocytes to vessel walls [16,17]. In the presence of an excess of reactive oxygen species, there is a reduction in the availability of endothelial NO, due to a greater formation of peroxynitrites, which induces many different damaging effects to lipid, protein, and nucleic acid metabolism. The decrease in NO availability induces different pro-atherogenic events, such as hyper-expression of vascular cell adhesion molecules (V-CAM; I-CAM), an increase in Hcy in "native" (and hence toxic) form, and a reduction in vasodilator response [17–19].

For these reasons, an assessment of pro- and antioxidant factors that are able to influence endothelial function, and particularly NO availability, seems relevant. In the present study of patients affected by coronary heart disease (CHD) and stratified by the presence/absence of smoking habit, we compared the flow-mediated dilatation (FMD) in response to reactive ischemia and the corresponding biochemical markers of both oxidative damage and antioxidant status, in order to establish which of these markers may be more useful for clinical, diagnostic use and control interventions [20].

2. Materials and methods

2.1. Patients

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants gave their written informed consent before the study.

Sixty subjects (all Caucasian) were recruited for the study. Thirty-two (aged 50±9 years, mean±SD, 13 females) were chronic cigarette smokers and 28 (aged 52±8 years, 15 females) were non-smokers. All of the subjects had previously been admitted to the cardiology unit of our institution and diagnosed as having coronary heart disease (CHD), ranging from acute myocardial infarction (AMI) to instable angina pectoris (IAP). Smoking habit (15–30 cigarettes daily) was the single or largely predominant risk factor in the first group; no other definite risk factors were present in the second group. No other disease (diabetes in particular) was observed in either group and all subjects had stopped using drugs (diuretics, nitrates, ACE inhibitors, when present, in some cases) in the last 48 h prior to the study. The dietary habits of the subjects are presented in Table 1.

2.2. Assessment of brachial arterial reactivity

A previously validated ultrasound study of brachial artery reactivity was performed in all patients upon entering the study and 6 months later using an Acuson 128 XP/10 mainframe (Acuson, Mountain View, California, USA) with a 7.0 MHz linear-array transducer. Images were stored on a super VHS videotape recorder for further analysis. The technique for assessing brachial artery FMD has already been described in detail elsewhere [21,22]. The methodology has an inter-observer variability in diameter measurements of 0.45±0.25%, yielding a coefficient of variation of 1.34% and a coefficient of repeatability of 0.8%. The study was performed early in the morning and the smokers were advised not to smoke.

2.3. Chemicals

All chemicals, of HPLC grade, were purchased from Farmitalia (Milan, Italy). Ascorbic acid, retinol, D-alpha-tocopherol, alpha-carotene, beta-carotene, tocopherol acetate, retinol acetate (the last two as internal standards),

Table 2
Main laboratory analytes in each group investigated

Groups	Age (years)	RBC×106	Hb g/dl	LDL mg/dl	HDL mg/dl	TGL mg/dl	TSL mg/dl	Glucose mg/dl	Creatinine mg/dl	Albumin g/dl
Non-smokers (n=28)	52±1.5	4.57±0.10	13.32±0.29	143.9±10.6	50.1±3.4	135.6±23.4	519±35	110.6±5.8	1.0±0.04	4.05±0.10
Smokers (n=32)	50±1.7	4.49±0.09	13.29±0.34	174.7±6.9	48.9±3.25	134.8±14.3	572±23	109.3±6.1	0.85±0.03	4.03±0.07

Mean±S.E.