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Reactive aldehyde-scavenging enzyme activities in atherosclerotic plaques of cigarette smokers and nonsmokers

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ABSTRACT

Objective: To investigate enzymatic reactive aldehyde-scavenging enzyme capacity together with lipid peroxidation as expression of oxidative stress in atherosclerotic plaques of cigarette smokers and nonsmokers. **Methods**: We have assessed specific enzymatic activities of class 1, 2, and 3 aldehyde dehydrogenase (ALDH1, ALDH2, and ALDH3, respectively), glutathione *S*-transferase (isozyme A4-4, GSTA4-4), and aldose reductase (AR), namely the major reactive aldehyde-scavenging enzymes, together with lipid peroxidation, i.e., fluorescent damage products of lipid peroxidation (FDPL), in carotid atherosclerotic plaques surgically removed from 17 cigarette smokers and 17 nonsmokers. **Results**: The enzymatic activities of ALDH1 plus ALDH2, ALDH3, GSTA4-4, and AR were significantly lower in the atherosclerotic plaques of smokers than in those of nonsmokers, while plaque FDPL levels were significantly higher in the smokers than in the nonsmokers. The amount of cigarette smoking was correlated inversely with the aforementioned plaque enzymatic activities and directly with plaque FDPL content. Plaque FDPL levels were inversely correlated with plaque enzymatic activities in smokers and nonsmokers. The degree of carotid atherosclerotic stenosis, as expression of atherosclerosis severity, was correlated inversely with plaque enzymatic activities and directly with plaque FDPL levels in smokers and nonsmokers; moreover, the degree of carotid stenosis was directly correlated with the amount of cigarette smoking. **Conclusion**: atherosclerotic lesions of cigarette smokers are endowed with a depressed enzymatic reactive aldehyde-scavenging capacity eventually favoring oxidative stress and the severity of atherosclerosis.

1. Introduction

Reactive aldehydes of lipid peroxidation are cytotoxic compounds involved in the pathogenesis of atherosclerosis [1,2]. Indeed, bifunctional, i.e., malondialdehyde (MDA), and monofunctional reactive aldehydes, such as alkanals, alkenals and hydroxyalkenals, especially 4-hydroxy-2-nonenal (4-HNE), have atherogenic properties. However, the cell is physiologically endowed with enzymatic defenses able to metabolize such reactive aldehydes. In particular, aldehyde dehydrogenase (ALDH) can catalyze the pyridine nucleotide-dependent oxidation of MDA and monofunctional reactive aldehydes to the corresponding carboxylic acids [2]. 4-HNE is metabolized by class 1 ALDH (ALDH1), which has especially a cytosolic localization and can metabolize also MDA and alkanals such as hexanal, besides the ethanol metabolite acetaldehyde. Class 2 ALDH (ALDH2), which is localized in the mitochondria and, especially, in the cytosol in the arterial tissue [3], can metabolize 4-HNE and MDA, as well as acetaldehyde and the drug nitroglycerin [2]. Monofunctional aliphatic aldehydes, such as alkanals, alkenals and hydroxyalkenals, are substrates for class 3 ALDH (ALDH3), which is a constitutive or inducible enzyme present

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in the cytosolic and microsomal fraction [2]. Other relevant enzymes involved in reactive aldehyde detoxification are especially glutathione *S*-transferase (GST), in particular the isozyme GSTA4-4, which catalyzes the conjugation of 4-HNE to GSH [4–6], and aldose reductase (AR), which operates reactive aldehyde reductive metabolism [7,8].

Cigarette smoking is a well-known risk factor for the development of atherosclerosis and may be responsible for accelerated atherosclerosis and cardiovascular disease [9,10]. Cigarette smoke (CS) contains a bewildering array of toxic compounds including reactive aldehydes and oxidant species [9], which can promote lipid peroxidation leading to generation of further amounts of reactive aldehydes with cytotoxic and atherogenic properties. Thus, it is possible that some mechanisms involving reactive aldehyde burden and impaired reactive aldehyde-scavenging enzyme activities may be implicated in the atherosclerosis of cigarette smokers. In the present study, we have assessed specific enzymatic activities of ALDH1 plus ALDH2, ALDH3, GSTA4-4, and AR, as well as lipid peroxidation as expression of oxidative stress, in human carotid atherosclerotic plaques surgically removed from cigarette smokers and nonsmokers. The results show that the atherosclerotic lesions of smokers are endowed with a lower enzymatic reactive aldehyde-scavenging capacity than those of nonsmokers eventually favoring oxidative stress and the severity of atherosclerosis.

2. Materials and methods

2.1. Study subjects

Carotid atherosclerotic plaques were surgically removed from a total of 34 patients (24 men and 10 women, age 69.2 ± 4.8 years, 17 smokers and 17 nonsmokers) scheduled for elective carotid artery endarterectomy. More specifically, we studied 17 consecutive active cigarette smokers comprising 12 men and 5 women (age 69.1 ± 4.7 years); seventeen nonsmokers (12 men and 5 women, age 69.3 ± 5 years) were selected to be matched for clinical characteristics with cigarette smokers (Table 1). Institutional review board approval was obtained for plaque procurement, and informed consent was given by all participants. As expected for endarterectomy specimens, plaques represented advanced fibrofatty lesions, without, however, significant thrombosis or calcification; in this regard, about 12% of patients, namely two cigarette smokers and two nonsmokers, were not considered in the study for the presence of significantly calcified carotid plaques as also assessed by carotid echo-color Doppler performed pre-operatively. As shown in Table 1, the two groups of cigarette smokers and nonsmokers were well balanced for the presence of the major cardiovascular risk factors arterial hypertension, diabetes mellitus, and hyperlipidemia. No patient in both group had inflammatory, neoplastic, hematologic, infectious, hepatic, and/or renal disease, nor was alcohol abuser or took antioxidant supplements; in particular, the number of patients referring alcohol consumption and the amount of alcohol intake (which was modest and essentially related to wine consumption) was similar in smokers and nonsmokers. Patients were of the same geographic area (Chieti, Abruzzo, Italy) and had a similar dietetic pattern. Substantially all patients took low-dose aspirin as antiplatelet agent (Table 1), while nobody took nitrates. Antihypertensive therapy, usually based on ACE-inhibitors, calcium antagonists, beta-blockers and/or diuretics, was similar in smokers and nonsmokers; diabetic patients were usually treated on a dietetic basis and/or with insulin. Seven smokers and eight nonsmokers had started statin treatment (Table 1). Cigarette smoking was assessed by asking subjects the number of packs per day and the number of years they had smoked; multiplication of the responses produced a mean ± SD value of 44.2 ± 11.5 pack-years of smoking.

Table 1
Clinical characteristics of cigarette smokers and nonsmokers.

	Smokers	Nonsmokers	*P*
n	17	17	
Age, years	69.1 ± 4.7	69.3 ± 5	NS
Sex, M/F	12/5	12/5	NS
BMI (Kg/m^2)	25.5 ± 3.3	25.8 ± 3.1	NS
Serum total cholesterol (mM)	5.3 ± 1.2	5.5 ± 1.3	NS
Serum triglycerides (mM)	1.6 ± 0.7	1.5 ± 0.6	NS
Hypertension, *n* (%)	10 (58.8)	11 (64.7)	NS
Diabetes mellitus, *n* (%)	4 (23.5)	4 (23.5)	NS
Hyperlipidemia, *n* (%)	6 (35.3)	7 (41.2)	NS
Antiplatelet drugs, *n* (%)	16 (94.1)	16 (94.1)	NS
Statins, *n* (%)	7 (41.2)	8 (47)	NS

Means ± SD, or number (*n*) and percentage (%) of patients. NS: not significant.

2.2. Biochemical analyses

Reagents of the highest purity were from Sigma–Aldrich Corp. (St. Louis, MO, USA).

Basically according to Koivula et al. [11], for enzyme activity determination a plaque portion was homogenized in ice-cold 10 mM potassium phosphate buffer, pH 7.4, plus 0.5 mM mercaptoethanol, 0.8 mM EDTA, and 0.1% sodium deoxycholate; then, centrifugation at 3000 × *g* at 4 °C for 25 min was performed, and the resulting supernatant used for assay of specific enzymatic activities. Another plaque portion was homogenized in butylated hydroxytoluene-containing ice-cold 20 mM Tris/HCl buffer, pH 7.4, followed by centrifugation at 1000 × *g* at 4 °C for 15 min; the resulting supernatant was used to assess lipid peroxidation, namely fluorescent damage products of lipid peroxidation (FDPL).

The activity of ALDH1 plus ALDH2 was determined by monitoring at 37 °C enzyme-dependent NADH formation from NAD^+ [11]. To increase assay sensitivity, NADH formation was assessed fluorometrically at 340/470 nm excitation/emission. The assay system contained 50 mM potassium phosphate buffer, pH 8.5, 1 mM NAD^+, 2 μM rotenone (to inhibit NADH consumption by complex 1 of the electron transfer chain), 5 mM pyrazole (to inhibith alcohol dehydrogenase), the homogenate supernatant, and 8 mM propionaldehyde, which is a preferred substrate for both class 1 and 2 ALDH.

ALDH3 activity was assayed as reported above for ALDH1 plus ALDH2, except for the use of 1.5 mM hexanal as the aldehydic substrate. Based also on our experience, use of NAD^+ as cofactor is most appropriate to determine specific enzymatic activity with hexanal as substrate. The activity of ALDH1 plus ALDH2 and of ALDH3 is expressed as nmol NADH formed/min (mU)/mg protein.

GSTA4-4 activity was measured basically as previously reported [4]. The assay system contained 0.1 M potassium phosphate buffer, pH 6.5, 0.5 mM GSH, the homogenate supernatant, and 20 μM *trans*-nonenal as substrate to start the reaction; the decrease in *trans*-nonenal-related absorbance at 225 nm was followed spectrophotometrically at 37 °C. Results were calculated as nmol *trans*-nonenal conjugated to GSH/min (mU)/mg protein, assuming a molar extinction coefficient for *trans*-nonenal of 19,200.

AR activity was assayed in a reaction system formed by 50 mM potassium phosphate buffer, pH 7.0, 100 mM ammonium sulfate, 30 μM NADPH, the homogenate supernatant, and 5 mM glyceraldehyde as substrate [12]. Enzyme-dependent oxidative consumption of NADPH was evaluated fluorometrically at 37 °C with excitation/emission wavelengths of 340/450 nm, expressing the results as nmol NADPH oxidized/min (mU)/mg protein.

For all enzymatic activities, two blanks were always considered, namely one with the enzyme source but without the substrate