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Original Article

Treatment with beta-blockers is associated with lower levels of Lp-PLA2 and suPAR in carotid plaques

Giuseppe Asciutto [a,*], Andreas Edsfeldt [b], Nuno V. Dias [a], Jan Nilsson [b], Cornelia Prehn [c], Jerzy Adamski [c,d], Isabel Gonçalves [b,e]

[a] Vascular Center Malmö-Lund, Skåne University Hospital, Malmö, Sweden
[b] Department of Clinical Sciences, Experimental Cardiovascular Research Unit, Clinical Research Center, Lund University, Malmö
[c] Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Genome Analysis Center, Neuherberg, Germany
[d] Chair of Experimental Genetics, Technische Universität München, Munich, Germany
[e] Department of Cardiology, Skåne University Hospital, Malmö

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ABSTRACT

Objectives: To determine whether a long-term treatment with beta-blockers influences the inflammatory activity in carotid artery disease by reducing the carotid plaque levels of lipoprotein-associated phospholipase A2 (Lp-PLA2), its enzymatic products lysophosphatidylcholine (lysoPCs), and of soluble urokinase plasminogen activator receptor (suPAR).
Materials and Methods: One hundred and thirty-four patients with significant symptomatic or asymptomatic carotid stenosis undergoing surgery were prospectively included and divided into two groups (Group A or B) based on the absence or presence of an on-going long-term oral treatment with beta-blockers. The harvested carotid plaques were analyzed for the levels of lysoPCs using mass spectrometry and Lp-PLA2 and suPAR by Enzyme-linked immunosorbent assay (ELISA).
Results: Plaques of patients on long-term treatment with beta-blockers revealed lower levels of Lp-PLA2 (Group A 0.752±0.393 ug/g vs. Group B 0.644±0.445 ug/g, P=.049) as well as suPAR (Group A 0.044±0.024 μg/g vs. Group B 0.036±0.025 μg/g, P=.028). Levels of Lp-PLA2 and suPAR were positively correlated (r=.637, P<.0001). Lp-PLA2 and suPAR levels were also correlated (P<.0001) with the three lysoPC species tested (lysoPC 16:0, lysoPC 18:0. lysoPC 18:1). All the above-mentioned findings were confirmed after correction for age, gender, hypertension, coronary artery disease, and statin usage.
Conclusions: The reduced levels of Lp-PLA2 and suPAR in human carotid plaques of subjects on long-term treatment with beta-blockers suggest their possible protective role in plaque inflammation. Our findings support an even more selective Lp-PLA2 and suPAR inhibition as a possible strategy for the prevention of cardiovascular disease.

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1. Introduction

Carotid endarterectomy (CEA) outcomes are influenced by multiple factors such as preoperative medical therapies [1].

In recent decades, multiple efforts have been carried out in order to reduce the risk of atherogenesis that showed to be triggered by an underlying inflammatory process [2,3]. Among the factors initiating the inflammatory response, which lead to the atherogenic process, a major role has been attributed to a high sympathetic nerve beta1-activity [4].

The laminar flow patterns produced by inhibiting the beta-adrenergic system in patients with carotid artery stenosis (CAS) [5,6] have been suggested as one of the mechanisms behind the protective effect of beta-blockers with respect to the development of carotid artery plaque.

The aim of this study was to investigate the influences of long-term treatment with beta-blockers beyond the ones attributed to their hemodynamic effect. More specifically, two potential biomarkers, which have shown to play a key role in regulating the inflammatory process leading to the atherogenesis such as soluble urokinase plasminogen activator receptor (suPAR) and lipoprotein-associated phospholipase A2 (Lp-PLA2), as well as its hydrolytic

Conflict of Interest Statement: All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

* Corresponding author. Faculty of Medicine, Lund University, Vascular Center Malmö-Lund, Skåne University Hospital, 205 02 Malmö, Sweden. Tel.: +46 40 338060; fax: +46 40 338097.
E-mail address: giuseppe.asciutto@med.lu.se (G. Asciutto).

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products lysophosphatidylcholines (lysoPCs), were analyzed in human carotid plaques and related to the current use of beta-blockers and the development of cerebrovascular symptoms. Our hypothesis was that long-term treatment with beta-blockers would decrease the expression of these potential markers of inflammation and, indirectly, the vulnerability of the carotid plaques.

2. Material and methods

Patients undergoing CEA at our vascular department between November 2005 and June 2008 were enrolled in this prospective study after giving informed consent. The study protocol was approved by the regional ethical committee. Information about comorbidities and past medical history were obtained through preoperative interviews, as well as the available medical records.

Cardiovascular risk factors such as hypertension (systolic blood pressure >140 mmHg), diabetes, coronary artery disease (CAD), smoking, dyslipidemia, immunological disorders, and body mass index (BMI) were recorded. Patients with ipsilateral carotid artery occlusion, radiation-induced CAS, or restenosis after previous CEA or endovascular treatment were excluded from the study cohort.

The regional medication register was analyzed to identify and determine the duration of an eventual ongoing treatment with beta-blockers. This is a health care database where all the prescriptions are registered. Long-term treatment was assumed in patients receiving beta-blockers of any type and dose for at least six months before surgery, regardless of the primary indication.

All patients underwent a standardized US examination of the carotid arteries the day before surgery and were clinically assessed by an independent accredited neurologist preoperatively. The indications for surgery were CAS associated with ipsilateral symptoms and a stenosis degree >70% or, in patients without neurological symptoms, a CAS degree >80%. The stenosis degree was assessed with ultrasound based on flow velocities as previously validated [7]. Patients were considered to have asymptomatic disease if they had had no *amaurosis fugax* (AF), transient ischemic attacks (TIAs), or strokes in the 6 months prior to surgery.

All patients undergoing CEA at our institution were routinely on long-term treatment with statins (simvastatin, 40 mg) and acetylsalicylic acid (75 mg) or clopidogrel (75 mg) daily. If no contraindications were present, patients not on long-term treatment with beta-blockers received a single dose of metoprolol succinate (50 mg) on the day before surgery and continued on this for the first 30 postoperative days and were defined as not on long-term treatment for the statistical analysis.

2.1. Sample preparation

The plaques removed by CEA were immediately snap frozen in liquid nitrogen. A 1-mm-thick fragment from the most stenotic region of the plaque was removed for histological examination. The remaining parts of the plaque were weighed, cut into pieces while still frozen, and homogenized as previously described [8].

2.2. LP-PLA2 assessment

Enzyme-linked immunosorbent assay (ELISA) was used for measuring Lp-PLA2 protein levels. Plaque homogenate (50 μl) were centrifuged at 13000 g for 10 min. The supernatant (25 μl) was then removed and used to measure Lp-PLA2. The procedure was performed according to the manufacturer's instructions (The PLAC Test ELISA Kit, diaDexus Inc, San Francisco, CA, USA). The Magellan V 6.4 program was used to measure LpPLA2 (absorbance, 450 nm) in a Sunrise ELISA reader (Tecan, Austria GmbH, Grödig, Austria).

2.3. Lysophosphatidylcholines assessment

Homogenates were analyzed as described for other tissues [9]. Precooled methanol (160 μL) was added to tubes containing frozen plaque homogenate (40 μL). Samples were homogenized further using a Precellys 24 homogenizer (PEQLAB Biotechnology GmbH, Erlangen, Germany). Homogenization was repeated three times for 20 s at 5500 rpm, with 30-s cooling intervals between the homogenization steps. Homogenates were then centrifuged (5 min at 10000 g, room temperature). Supernatants (20 μL) were analyzed for lysoPC 16:0, lysoPC 18:0, and lysoPC 18:1 using Absolute*IDQ* p150 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria). Further details on quantification and assays on API 4000 mass spectrometer (AB Sciex, Darmstadt, Germany) have been previously described [9,10].

2.4. Measurement of suPAR in human carotid plaques

Measurement of suPAR levels in carotid plaque homogenates were performed using suPARnostic Standard ELISA Kit (ViroGates, Birkerød, Denmark) with suPAR standards (1.0–20.7 ng/mL) and a blank control (2.6–3.4 ng/ml). Samples were mixed with peroxidase-conjugate monoclonal mouse anti-suPAR antibody and added to a clear microwell plate that was precoated with a monoclonal rat antihuman suPAR antibody. Then 3,3′,5,5′-tetramethylbenzidine was added to the wells, and it was incubated for 20 min in the dark. The absorbance at 450 nm, reference filter 650 nm, was measured with TECAN sunrise Absorbance Reader, Magellan, version 6.4 (Tecan, Austria GmbH, Grödig, Austria). The concentrations were determined by interpolation on the standard curve.

2.5. Statistical analysis

LysoPC's, Lp-PLA2, and suPAR have been normalized to the wet weight of the plaque. Continuous variables are presented as mean (standard deviation, S.D.) when not stated otherwise, while categorical variables are presented as percentages. Pearson's chi-square is used for categorical variables. Student's *t* test is used for continuous variables whenever normally distributed, while Mann–Whitney *U* test was used for nonnormally distributed variables.

Pearson's correlation is used for normally distributed variables, while Spearman's rank correlation is used for nonnormally distributed variables. Simple and multiple linear regressions are used to explore the relationship between two or more variables.

Correction for usual atherosclerosis risk factors as well as for statin usage was done. Information about comorbidities was incomplete only for the occurrence of inflammatory diseases and immunotherapy.

A P value of <.050 was considered statistically significant. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) 19.0 (SPSS Inc, Chicago, IL, USA).

3. Results

One hundred and thirty-seven plaques from 134 patients (68±9 years, 93 males) undergoing CEA (bilateral in three cases) were included. Seventy-eight (57%) plaques were associated with ipsilateral hemispheric symptoms while 59 (43%) were not.

The study cohort was divided into two subgroups based on the absence (Group A, n=78 plaques) or presence (Group B, n=59 plaques) of long-term treatment with beta-blockers.

As shown in Table 1, Group B patients had more frequently known CAD (P<.0001), as well as arterial hypertension (P<.0001), higher BMI (P=.034), and simultaneous treatment with statins (P=.003).

Median age (P=.914), the degree of stenosis (P=.909), incidence of preoperative neurological symptoms related to the operated CAS (P=.110), and the time between neurological symptoms and CEA (P=.410) were not different in the two groups.

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