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Plasma annexin A5 level relates inversely to the severity of coronary stenosis

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Abstract

Exogenous radiolabeled annexin A5 is taken up by atherosclerotic tissue. We measured endogenous plasma annexin A5 and circulating oxidized low-density lipoprotein (oxLDL), a biochemical marker of atherosclerosis, in men with either severe angiographically determined coronary stenosis ($n = 90$) or no or only minor stenosis ($n = 96$). Men without history of cardiac disease or treatment and free of plaques in the carotid artery (by ultrasonography) were taken as controls ($n = 87$). Opposite to oxLDL, annexin A5 decreased at increasing severity of stenosis. OxLDL was lowest and annexin A5 was highest in controls. Percentage differences between groups were higher for annexin A5 than for oxLDL, and highest for oxLDL/annexin A5 ratio. The oxLDL/annexin A5 ratio is a better marker of the severity of coronary stenosis than oxLDL alone, may reflect the presence and extent of the atherosclerotic cardiovascular disease, and might prove useful for preclinical screening purposes.

Keywords: Angiography; Annexin A5; Atherosclerosis; Cardiovascular diseases; Coronary disease; Coronary stenosis; Oxidized phospholipids; Oxidized low-density lipoprotein

Annexins comprise a group of evolutionary highly conserved proteins with calcium and membrane binding properties. One of the 12 annexins found in vertebrates, classified as the annexin A subfamily, is annexin A5. It is widely distributed intracellularly in human tissues [1] and released upon (traumatic) injury [2,3]. Because annexin A5 forms highly ordered two-dimensional crystals that coat the external leaflets of phospholipid bilayers, it can shield negatively charged phospholipids exposed by activated and/or apoptotic cells from availability for critical phospholipid-dependent coagulation reactions [4]. Atherosclerosis is a chronic inflammatory disease of the vessel wall, characterized by the local presence of increased amounts of activated and apoptotic cells. In addition, atherosclerotic plaques contain high amounts of oxidized low-density lipoprotein (oxLDL) which, in contrast to native LDL, specifically binds annexin A5 [5]. OxLDL may be formed by oxidative processes during migration of the LDL particles in the vessel wall [6,7] and is a prerequisite for atherogenesis. Whereas most of the oxLDL is retained in the vessel walls, part of it may re-enter the circulation and the plasma level of oxLDL has been shown to be a useful marker for identifying patients with coronary artery

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disease and persons at high cardiovascular risk [8–12]. In agreement with an elevated inflammatory state and accumulation of oxLDL, high amounts of annexin A5 are found in atherosclerotic plaques [1,13]. Moreover, recently, it was found that exogenous radiolabeled annexin A5 is taken up by aortic tissue and can be used for the non-invasive detection of apoptosis in animal models of human atherosclerosis [14,15] and for the identification of instability of atherosclerotic plaques in man [16]. The relation of stable atherosclerotic cardiovascular disease with plasma levels of endogenous annexin A5, however, is essentially unexplored. It has been shown that acutely after myocardial infarction, plasma level of annexin A5 is elevated due to release from cardiomyocytes [2]. In plasma of the healthy population annexin A5 is present in low ranges of 0.6–28 μg/L [17]. We hypothesize that due to binding of annexin A5 to components of the atherosclerotic plaque, the plasma level of endogenous annexin A5 is inversely associated with the presence and extent of atherosclerotic plaque formation and may have diagnostic value. Because coronary angiography is currently still the most used method in every-day practice to diagnose coronary heart disease, we investigated plasma levels of annexin A5 in relation to angiographically determined coronary stenosis.

Methods

Subjects. We used plasma samples from a study that was conducted previously in several hospitals and clinical centers in Rotterdam and Dordrecht in The Netherlands, to investigate coronary atherosclerosis in relation to markers of LDL oxidation [18]. From 1993 to 1995, from 1489 men undergoing a first coronary angiography, fasting venous blood had been collected within 2 months after coronary angiography. Seven hundred and eighty nine of them met the inclusion criteria (45–80 years old, alive, no myocardial infarction in the year before the study, no diabetes mellitus type 1 or 2, no liver, kidney or thyroid disease, no alcohol or drug abuse, no use of HMG-CoA reductase inhibitors (statins), no psychiatric complaints and not a vegetarian) and were willing to participate. Subjects were selected who had severe coronary stenosis (>85% stenosis in one and >50% stenosis in a second major coronary vessel, cases) and subjects with no or minor coronary stenosis (a maximum of 50% stenosis in no more than two of the three major coronary vessels and less than 10% stenosis in the third coronary vessel, hospital controls). This categorization was based on a clinical rationale: subjects with severe coronary stenosis (according to the criteria above) generally receive additional interventional treatment (bypass, angioplasty), but stenosis below 50% (in maximally two of the three major coronary vessels) is mostly left without intervention, except for treatment of cardiovascular risk factors. Two hundred and seventy-nine men fulfilled the stenosis criteria, but because only from 90 cases and 96 hospital controls plasma samples were available that had not been thawed since the time of blood collection, 186 patients entered the present study.

Plasma samples from population control subjects were obtained from The Rotterdam Study, a population-based cohort study on chronic and disabling diseases (conducted from 1991 to 1993) [19], and by additional recruitment (performed in 1994) of men between 45 and 55 years of age [18]. Thus, all population controls were recruited in the same region and the same period as the hospital subjects. No angiographic data were available, but the carotid artery vessel walls were examined by ultrasound echography. The subjects who were selected had no plaques in the carotid artery and no history of cardiac disease or treatment. After exclusion of subjects who met one or more of the exclusion criteria of the present study (female, diabetes mellitus 1 or 2, liver, kidney or thyroid disease, use of statins, vegetarian, and alcohol or drug abuse) and matching to hospital subjects with respect to smoking habits (smoking and non-smoking), we obtained 87 population control subjects of whom plasma samples had not been thawed in between.

All blood samples had been taken in EDTA Vacutainer tubes and placed on ice immediately. Within 1 h after blood collection plasma was prepared by centrifugation at 1750*g* for 15 min, frozen with saccharose (10%, w/v) in liquid nitrogen, and stored at −80 °C.

We obtained medical histories from medical files and through a questionnaire administered at the time of blood sampling. Also information on smoking and drinking patterns, drug use and family history of cardiovascular disease were available. Weight, height, and blood pressure (measured twice at one occasion in sitting position at the right upper arm with a random-zero sphygmomanometer) had been measured. Hypertension was defined at that time as a systolic blood pressure of 160 mmHg or more or a diastolic blood pressure of 95 mmHg or more, or use of antihypertensive drugs. An Ethics Committee on Human Research approved the study, and all participants gave informed consent.

Biochemical measurements. Plasma cholesterol and triglycerides were determined using commercially available reagents (Boehringer-Mannheim, Mannheim, Germany). Hypercholesterolemia was defined as plasma total cholesterol >6.5 mmol/L or use of cholesterol lowering medication. HDL-cholesterol was determined with the phosphotungstate/Mg^{2+} method [20]. LDL-cholesterol was calculated with the Friedewald formula. Apolipoprotein B (apoB) was quantified by immunonephelometry [21]. Plasma lipoprotein(a) was measured by an in-house ELISA using purified IgG polyclonal antibodies to apolipoprotein(a) developed in a rabbit [22]. Intra- and interassay coefficients of variation of the assay amounted 3.1% and 8%, respectively. For measurement of oxLDL a commercial non-competitive ELISA (Mercodia, Uppsala, Sweden) was used. The assay uses monoclonal antibody 4E6 to specifically capture oxidized apoB from the sample, which is subsequently detected with an antibody to apoB. Intra- and interassay coefficients of variation of the assay amounted 6% and 7%, respectively. As shown previously by our group [23] and confirmed recently by Holvoet et al. [24], oxLDL can be measured in EDTA plasma stored for longer periods (more than 15 years) at −80 °C, provided that the samples had been collected carefully and had not been thawed. Plasma annexin A5 and high-sensitivity C-reactive protein (hsCRP) were measured with commercial enzyme immunoassays (Hyphen BioMed, Neuville-sur-Oise, France, and Dako, Glastrup, Denmark, respectively) according to the instructions of the manufacturers. Intra- and interassay coefficients of variation amounted 1.4% and 3.8% for annexin A5 assay and 6.0% and 9.7% for hsCRP assay, respectively. Because all samples had not been thawed since time of blood collection, hsCRP could be measured without reservation; annexin A5 is insensitive to storage conditions.

Because we have previously shown that annexin A5 at physiological concentrations binds to oxLDL, we examined whether this binding of annexin A5 to oxLDL affects the measurement of oxLDL, and reversely, whether the presence of oxLDL influences the detection of annexin A5. We found that addition of annexin A5 (0.2–192 ng/ml, final concentration) did not influence the detection of 6.5 mU/L oxLDL in plasma, and addition of oxLDL (25–100 U/L, final concentration) did not influence the detection of 5 ng/ml annexin A5 in plasma. This suggests that the measurement of annexin A5 is not disturbed at increased plasma levels of oxLDL, and that elevated levels of annexin A5 do not disturb the detection of oxLDL.

Statistical analysis. The Mann–Whitney test was used to compare between group differences in proportions. Between group comparisons of continuous variables were performed with Student's *t*-test for unpaired samples. For triglycerides and time of cardiac complaints natural log-transformed data were tested. For hsCRP, lipoprotein(a), oxLDL, and annexin A5 age-standardized ln-values were calculated and tested. To standardize for age, the predicted value of a variable was calculated by linear regression and subtracted from the measured value to which the overall mean value of the variable was added. For presentation, mean and 95% CI of ln-values were converted to normal. A p-value < 0.05 was considered statistically significant. Analyses were performed using SPSS 12.01 for Windows.