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Urinary cysteinyl leukotrienes in one-year follow-up of percutaneous transluminal angioplasty for peripheral arterial occlusive disease

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ABSTRACT

Background and aims: Treatment of severe peripheral arterial occlusive disease requires percutaneous revascularization. However, little is known about risk factors or predictors for reocclusion/restenosis. Cysteinyl leukotrienes are highly bioactive lipid mediators of inflammation. Their intravascular production may take place in the atheromatous plaque or result from interaction within activated leukocyte-platelet aggregates.

Methods: We prospectively measured urinary leukotriene E_4, the main end-metabolite of cysteinyl leukotrienes in a group of 179 subjects with peripheral artery occlusive disease of the lower extremities. At the enrollment to the study, 22.9% had angioplasty and the remaining had angioplasty with stent implantation. During 12-month follow-up, 29.6% developed reocclusion/restenosis despite a standard pharmacotherapy. We evaluated treatment outcomes at 1, 3, 6 and 12-month follow-up visits, along with urinary leukotriene E_4 excretion.

Results: During the study period, we observed a linear increase of urinary leukotriene E_4 excretion only in subjects whose lower limb ischemia worsened. Moreover, elevated leukotriene E_4 in urine was found only in subjects who developed reocclusion/restenosis. This was significant not only as a coincidence at the time of the follow-up visit, but leukotriene E_4 elevation preceded clinical manifestation of reocclusion/restenosis.

Conclusions: Our results demonstrated that serial measurements of urinary leukotriene E_4 allowed to predict failure of angioplasty with/or without stent implantation for peripheral artery occlusive disease. However, to prove causality between cysteinyl leukotrienes overproduction and occlusive lower limb ischemia, a clinical trial with leukotrienes modifying drugs would be required.

1. Introduction

Atherosclerosis is an inflammatory vascular disease of arteries [1] increasing its prevalence in developed countries. Endothelial dysfunction is the hallmark of the disease and useful marker of the onset of vascular complications in diabetes or dyslipidemias. Serum levels of high sensitivity CRP, interleukine-8 or VCAM-1 are elevated, whereas antioxidant capacity is lowered. It has been recently demonstrated that endothelial cells exposed to the serum of peripheral artery disease patients has much higher apoptosis rate and generate much more reactive oxygen species [2]. Among numerous inflammatory cells and their mediators, there are leukotrienes, a class of lipid mediators of inflammation well studied in allergic disorders. Leukotrienes are products of enzymatic oxidation of polyunsaturated acids. 5-lipoxygenase [5-LO] is responsible for a stereospecific dehydrogenation and subsequent deoxygenation of polyunsaturated fatty acids, mostly arachidonic, released from the cell membrane during inflammation [2]. 5-LO, originally identified in polymorphonuclear leukocytes [3], is expressed in

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other myeloid-lineage cells and is inducible in endothelial cells. Overexpression of 5-LO was demonstrated in macrophages, dendritic cells, foam cells, mast cells and neutrophils within atherosclerotic vessels. The product of this enzyme is an unstable epoxide intermediate leukotriene A_4 (LTA_4), metabolized subsequently to LTB_4 or LTC_4 and other cysteinyl leukotrienes. Drugs modifying the 5-LO pathway were evaluated in asthma and other allergic disorders [4]. However, a growing understanding of the role of inflammation widened attention to the potential role of leukotrienes and their metabolism in atherosclerosis.

In 1999, Cyrus et al. observed that 12/15-lipoxygenase (*12/15-LO*) knockout decreased atherogenesis in *apoE*-deficient mice [5]. The role of the enzyme in the formation of atherosclerotic plaque was confirmed by them, suggesting oxidized LDL particles entry into the subendothelial space as the stimulus for the inflammation. In 2002, Mehrabian et al. identified 5-LO as a crucial enzyme contributing to atherosclerotic susceptibility in mice [6]. This observation, after validation in humans [7], has focused the research on the role of leukotrienes in the pathogenesis of atherosclerotic plaque [8]. It was speculated that leukotriene modifying drugs used in the treatment of asthma could have beneficial effects in limiting atherogenesis [9]. Aiello et al. showed that LTB_4 receptor antagonism reduced monocytic foam cells formation in mice [10]. Lotzer et al. demonstrated that macrophage-derived LTs differentially activate CysLTR1 receptor by paracrine stimulation and CysLTR2 by autocrine and paracrine stimulation during inflammation and atherogenesis [11]. The results of the study by Jawien et al. showed that the inhibition of 5-LO activating protein (FLAP) by MK-886 or BAYx1005 significantly prevented the development of atherosclerosis in *apoE*/*LDLR*-double knockout mice. Moreover, the study showed that CysLTR1 blocker montelukast decreased atherosclerosis in these mice [12–14].

Previous clinical studies revealed that urinary LTE_4 concentrations reflect systemic production and concentration of this metabolite. Urinary leukotriene E4 ($uLTE_4$) was validated as a useful biomarker of the systemic CysLTs biosynthesis in allergic disorders (for Review see Ref. [15]). Excretion of uLTE4 can fluctuate as demonstrated by almost tenfold rise within a few hours after asthmatic attack precipitated in the drug hypersensitive subjects or a 30% drop within 3 days after smoking cessation [16].

Previous studies demonstrated both local elevations of leukotriene C4 concentration in coronary sinus [17] and increased concentrations of urinary LTE4 in coronary artery disease patients [18] – especially during documented episodes of ischemia (unstable angina and myocardial infarction). Szczeklik et al. [19] investigated uLTE4 levels in peripheral artery disease and aortic aneurysm patients establishing both baseline concentrations and changes following surgical revascularization procedures.

Therefore, we also wondered how endovascular revascularization procedures affect systemic biosynthesis of CysLTs in atherosclerotic patients with critical stenosis or occlusion of lower limbs arteries. The study was designed to compare urinary excretion of leukotriene E_4 ($uLTE_4$), the ultimate metabolite of CysLTs before and after percutaneous transluminal angioplasty (PTA) with a follow-up period of 12 months.

2. Materials and methods

In this study, we enrolled consecutive patients with peripheral arterial occlusive disease (PAOD) who required PTA in either the iliac, femoral, or popliteal arteries and met all inclusion and exclusion criteria. The entry criterion for inclusion was severe claudication and resting pain. Patients with signs of inflammation, trophic changes, ulcerations, asthma, severe chronic kidney disease, neoplastic disease were excluded. Thus, the group studied corresponded to stage 3 or 4 of PAOD using Rutherford's classification. All subjects in this study received an antiplatelet aspirin (75 mg) as well as a statin (atorvastatin or rosuvastatin 20–40 mg) throughout the whole study and clopidogrel for 6 weeks after PTA. For most of the patients, statin therapy was a long-term medication and dosage remained unchanged throughout the follow-up period. Inclusion and exclusion criteria are listed in Table 1. All patients signed an informed consent to participate, and the local Jagiellonian University Ethics Committee accepted this study.

Excretion of $uLTE_4$ was measured in urine samples by high-performance liquid chromatography-mass spectrometry and recalculated to the creatinine concentration. Urine samples were collected the morning before PTA (T_0), immediately following PTA (T_1), and the next day after PTA (T_2). After that, all the study subjects were observed for 12 months. Their urine samples were collected during follow-up visits, and $uLTE_4$ was measured at 1 month (T_3), 3 months (T_4), 6 months (T_5) and 12 months (T_6) after PTA. Except the sample collected immediately after PTA, all other samples were collected in the morning. Samples were immediately transferred to the laboratory facility within the same building. During the next 30 min, we separated urine sample from the sediment by centrifugation (10 min, 5000*g*), and prepared 1 ml aliquots from supernatant and placed them in Eppendorf microcentrifuge tubes and transferred to the −70 °C freezer. All samples were analyzed as one batch. Previous tests confirmed the stability of uLTE4 in deep-frozen samples up to 24 months. The method for $uLTE_4$ measurement was previously validated and compared with the commercial enzyme-linked immunoassay (ELISA) in a population of asthmatics [20].

During the follow-up visits, ischemia was evaluated by Rutherford's scale, ankle–brachial index (ABI), and examination of arteries using USG with color Doppler. For assessment of restenosis/reocclusion, a compound parameter of decreased ABI and confirmatory USG imaging with color Doppler was used. Visits were scheduled at 1, 3, 6 and 12 months after the procedure. Patients' data were consistently recorded in the central database. This follow-up protocol is a standard of care in our center and was described previously [21].

3. Statistical calculations

Descriptive statistics were calculated as well as the arithmetic mean and standard deviation or median with the interquartile range depending on data distribution. Urinary excretion of $uLTE_4$ does not follow a normal distribution; thus, data were log-transformed to stabilize their variance. Measurements of $uLTE_4$ in samples collected at the control visits 1, 3, 6 and 12 months after revascularization were analyzed by a repeated measure ANOVA to evaluate their change over time in the perspective of the revascularization outcomes after 12 months. A logistic regression model was used to evaluate the correlation between $uLTE_4$ and restenosis/reocclusion. The model included the following confounding variables: gender, age, duration of ischemic symptoms, the presence of hypercholesterolemia, hypertension, heart failure, coronary artery disease, tobacco smoking status (in pack-years) and the presence of diabetes. A measurement of $uLTE_4$ in each time point of the study was used as a predictor for a binary outcome variable (i.e. restenosis/reocclusion yes/no). The goodness of fit of the model was estimated by a receiver operator characteristics (ROC) Odds ratio (OR) for each independent variable in the model was calculated along with a 95% confidence interval (95%CI). Log-rank test compared Kaplan-Meier estimator curves for restenosis/reocclusion events and groups.

Results with type I statistical error less than 0.05 were assumed significant. All calculations were done using Statistica for Windows