INTRODUCTION

Leukotrienes (LTs) are highly bioactive lipid mediators produced from polyunsaturated fatty acid - arachidonic acid (AA) - by activated leucocytes in the 5-lipoxygenase (5-LO) pathway. Cysteinyl-LTs (Cys-LTs) are products of glutathione coupling with leukotriene A4 and were investigated mostly in allergic disorders and inflammation (1, 2). The role of Cys-LTs in the development of atherosclerosis and its complications is well established (3, 4), and some genetic variants that increase the 5-LO activity predispose to myocardial infarction (MI) (5). Increased LTs production, and their synthesis pathway mRNA levels, have been found in biopsies from abdominal aortic aneurysms suggesting their possible role in the pathogenesis of this disease. There are at least two cysteinyl-leukotrienes receptors highly expressed in the heart, and leukotrienes influence the cardiac conduction system (6, 7). Based on experimental murine models, LTs influence also the fibroblast activation and local perfusion, which have important impact on the wound healing and later on the remodelling process of the scar tissue (8, 9). Cys-LTs are synthesized de novo from cell membrane phospholipids in response to specific pro-inflammatory signals (2). As strong mediators of vascular leak and smooth muscle constrictors, Cys-LTs may play a more general role in patients undergoing surgery.

Systemic production of Cys-LTs is well reflected by LTE4 concentration in urine (2, 10). A convenient and non-invasive method of enzymatic immunoassay for urinary LTE4 excretion correlates satisfactory with more sophisticated measurements, i.e. liquid chromatography-mass spectrometry (11).

The aim of the current study was to evaluate perioperative biosynthesis of LTE4 in noncardiac vascular surgery patients, and its impact on patients’ outcomes. To the best of our knowledge, no such a study was published so far.

MATERIAL AND METHODS

Study population

We enrolled 34 consecutive patients aged 50 years old or more admitted the Department of Vascular Surgery at St. John Grande Hospital in Cracow for elective surgery under general anaesthesia. Indications for surgery were either abdominal aortic aneurysm (AAA) or peripheral artery disease (PAD). All the study subjects gave their informed written consent and the study protocol was accepted by Bioethical Committee of the Jagiellonian University in Cracow. Exclusion criteria for recruitment were: glomerular filtration rate (GFR) out of the
range from 30 to 130 mL/min/1.73m² (n=3), early discharge from the hospital following the surgery resulting in incomplete urine sampling (n=1), and active inflammatory process confirmed by clinical status and laboratory markers (n=2). Thus, 28 patients were studied eventually, 6 of whom had AAA and the others had PAD.

Patients were followed-up for 30 days after surgery by a medical professional. Data on a composite major adverse cardiac events (MACE) were reported, which included: death from any cause, nonfatal cardiac arrest, myocardial infarction (MI), and stroke. During the first 3 days after the surgery patients were daily screened for troponin T elevation (4th generation assay, Roche Diagnostics) at the same time points when urine samples were collected for LTE4 measurements.

Leukotrienes measurements

Urinary leukotriene E₄ (uLTE₄) was studied in the urinary samples collected: on admission prior to surgery (LT₀), 6 hours postoperatively (LT₁), and as morning urine samples on the first (LT₂), second (LT₃) and third (LT₄) days after surgery. uLTE₄ was measured using ELISA kit (Cayman Chemical Co., Ann Arbor, Michigan, United States) following appropriate (1:10 to 1:30) dilution in phosphate-buffered saline (11). The results were adjusted for urinary creatinine concentration and were reported in picograms per milligram of creatinine.

Statistical analysis

Categorical variables were presented as number (percentage) whereas continuous variables were reported as median with interquartile range. Urinary leukotriene E₄ data were log-transformed to approximate normal distribution and the assumption was verified using Kolmogorov-Smirnov test. Urinary leukotriene E₄ measurements were compared using one way analysis of variance (ANOVA) for repeated measurements and post-hoc Tukey test was used for planned comparisons. Hypothesis on correlation between uLTE₄ and MACE outcomes 30 days after the surgery was tested using the logistic regression.

RESULTS

Characteristics of the study population are shown in Table 1. On the first day after the surgery, 3 patients had myocardial infarction (MI). No other MACE were observed during the 30-day observation period after the surgery.

Urinary leukotriene E₄ excretion raised in the first urine sample after surgery (LT₁) as compared to preoperative baseline value (LT₀) and remained elevated in the consecutive two samples after surgery (composition of mean LT₁ and LT₂ vs. LT₀; P=0.008). uLTE₄ returned to the baseline value on the second day after surgery (LT₃). No further change in uLTE₄ was noted on the following day (LT₄). uLTE₄ values and their changes are summarized in Table 1 and plotted on Fig. IA.

The baseline uLTE₄ (LT₀) correlated with the GFR (rho=0.43; P=0.03). A trend for higher baseline uLTE₄ (LT₀) excretion was among younger patients (rho=–0.36; P=0.064). No such correlations were observed in uLTE₄ excretion in the postoperative period (LT₁-LT₄). There was no influence of the gender, surgery indication (AAA or PAD), or comorbidities on uLTE₄.

Patients with MI during postoperative period tended to have increased baseline LTE₄ before surgery (LT₀; P=0.07) and this difference was present also after the surgery. Due to a limited number of these events (n=3) we compared repeated measurements ANOVA on 5 consecutive uLTE₄ measurements, which showed a highly significant elevation of this Cys-LTs among subjects with MI (P=0.006). The best discriminative

Table 1. Characteristics of the study patients group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male/Female ratio</th>
<th>Age (years)</th>
<th>Indication for surgery (AAA/PAD)</th>
<th>CAD history</th>
<th>MI history</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Current tobacco use</th>
<th>History of tobacco use</th>
<th>BMI</th>
<th>GFR</th>
<th>LT0</th>
<th>LT1</th>
<th>LT2</th>
<th>LT3</th>
<th>LT4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female ratio</td>
<td>21 (75%) / 7 (25%)</td>
<td>61.5 (59-72.5)</td>
<td>Indication for surgery (AAA/PAD)</td>
<td>6 (21.4%) / 22 (78.6%)</td>
<td>13 (46.4%)</td>
<td>9 (32.4%)</td>
<td>20 (71.4%)</td>
<td>4 (14.3%)</td>
<td>10 (35.7%)</td>
<td>26 (92.9%)</td>
<td>24.6 (21.2-27.6)</td>
<td>82.06 (72.6-98)</td>
<td>817 (390-1684)</td>
<td>1638.5 (826-3622.5)</td>
<td>1146 (440-1459)</td>
<td>650.5 (430-1052)</td>
</tr>
</tbody>
</table>

Data expressed as n (%) or median (25–75 interquartile range).
AAA - abdominal aortic aneurysm; PAD - peripheral artery disease; CAD - coronary artery disease; MI - myocardial infarction; BMI - body mass index; GFR - glomerular filtration rate (in mL/min/1.73 m²); LT₀-LT₄ - consecutive urinary leukotrienes E₄ measurements (in picograms per milligram of creatinine). Between variables correlation was analyzed using Pearson’s or Spearman’s rank correlation, as appropriate for the data distribution. All statistical analyses were performed using STATISTICA 10.0 software (StatSoft Inc. Tulsa, Ok., USA) with statistical type I error P<0.05 considered significant.
between MI and no-MI were morning urine samples collected on the first and second day after the surgery (post-hoc P=0.038 (L2) and P=0.017 (L3), Fig. 1B).

No correlation between uLTE 4 in the postoperative period and serum TnT were observed.

DISCUSSION

In this preliminary study we investigated a pattern of Cys-LTs biosynthesis in noncardiac vascular surgery patients during the perioperative period. Urinary leukotriene E 4 raised shortly after surgery and returned to the preoperative level on the second day. This increase in uLTE 4 excretion after the surgery is most probably due to enhanced biosynthesis of cysteinyl leukotrienes caused by a surgical insult on tissues and may suggest a possible role of Cys-LTs in the wound healing. A similar pattern of immediate post-operative increase in Cys-LTs was observed by Pillai et al. (12) in peripheral blood plasma samples, which was accompanied by increase of other lipid mediators. In current study, uLTE 4 correlated with patients kidney function. This is possibly a property of urinary excretion, an alternative to the biliary clearance, in which clearance of the end metabolite of Cys-LTs depends on the glomerular ultrafiltration. We were not able to confirm differences in the uLTE 4 dependent on age or sex, as described in other studies (13), however the current study group consisted predominantly of men in a similar age and limited number of patients was the main limitation of this study.

During the postoperative period we identified MI in 3 subjects. Interestingly, their uLTE 4 tended to be elevated, even prior to the surgery. Although this difference did not reach the statistical significance for a single point of time measurements, repeated measurements ANOVA on 5 urine samples showed highly significant elevation in uLTE 4 excretion among subjects with MI. A transcellular biosynthesis of Cys-LTs seems to contribute to the systemic production of these mediators. Circulating platelet-leukocyte aggregates are predictive for arterial thrombosis in subjects with atherosclerosis (14). It is plausible that uLTE4 might reflect a circulation level of by platelet-leukocyte aggregates, thus subjects with elevated uLTE4 could benefit from a more intense anti-platelet therapy protecting against MACE during post-operative period.

The limitation of this study was a small number of patients enrolled whereas incidence of MACE during postoperative period was in agreement with other studies (15, 16). However, a coincidence between elevation of uLTE 4 and perioperative MI warrants further investigations both on a predictive value of this parameter and a general contribution of systemic Cys-LTs biosynthesis to the cardiovascular risk. This pilot study also defines time points during the perioperative observation, suitable the best for uLTE4 monitoring in the future studies.

Acknowledgments: This work has been supported by the grant from the Polish Ministry of Science and Higher Education N N402 083939.

Conflict of interest: None declared.

REFERENCES


Received: June 3, 2014
Accepted: August 30, 2014

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