PHARMACOLOGICAL INHIBITION OF LEUKOTRIENE BIOSYNTHESIS: EFFECTS ON THE HEART CONDUCTANCE

Leukotrienes are lipid mediators produced via 5-lipoxygenase pathway of arachidonic acid. At least two cysteinyl-leukotrienes receptors are highly expressed in the heart, including the conduction system. Coronary angiography or angioplasty is accompanied by release of cysteinyl leukotrienes into coronary circulation and into urine. We tested the hypothesis that inhibition of leukotrienes biosynthesis would affect the conductance system function. In a double-blind placebo controlled study, patients with stable angina undergoing elective coronary catheterization or angioplasty were randomly assigned to 48 hrs treatment with a 5-lipooxygenase inhibitor (n=54) or placebo (n=49). ECG Holter recording was carried out for 24 hrs before and after the procedure and urinary leukotriene E4 measurements were done. Inhibition of 5-lipooxygenase caused 26% reduction of urinary leukotriene E4, associated with: 1) decrease in heart rate by about 7%, 2) enhanced heart rate variability; 3) protection against depressions in atrioventricular conductance and ventricular repolarization induced by the procedure. No effects on either arrhythmias, or ECG patterns of ischemia were noted. We conclude that pharmacological inhibition of 5-lipooxygenase, shortly before percutaneous coronary intervention, reveals specific actions of leukotrienes on the heart rhythm. Inhibitors of 5-lipooxygenase might be of interest as a novel class of cardiac drugs affecting the conductive system.

Key words: leukotrienes, heart rate variability, conductance system, 5-lipoxygenase, coronary arteries disease
**MATERIAL AND METHODS**

**Subjects**

The study group comprised 103 subjects, recruited from 348 consecutive patients with chronic stable angina, scheduled for elective coronary arteriography or coronary angioplasty between November 2006 and December 2007 in our Department. Exclusion criteria were: age under 30 or over 70 years, acute coronary events in the three months preceding the study, atrial fibrillation, pacemaker implantation, bronchial asthma or chronic obstructive pulmonary disease, renal or hepatic impairment, or symptoms of acute infection. Baseline patients’ characteristics are presented in Table 1. Informed consent was obtained from all the subjects. The study was accepted by the Ethics Committee of Jagiellonian University Medical College, Krakow.

**Study protocol**

Randomization was performed on admittance and the code was not broken until the final analyses. Due to exclusion criteria, ultimately 54 patients were assigned to treatment with zileuton (Zyflo, purchased from Cornerstone Therapeutics Inc., Cary, NC USA) and 49 to placebo. Study medication was given 600 mg p.o. every 6 hours; 24, 18, 12, 6 hours before and 1, 6, 12, 18 hours after coronary angiography or angioplasty. This dosage regimen has been recommended by the manufacturer for treatment of bronchial asthma and was demonstrated to effectively inhibit 5-lipoxygenase **ex vivo**. Urinary samples for measurements of uLTE4 were obtained 24 and 2 hours before, as well as 2 and 24 hours after coronary procedures. ECG Holter recording was done during 24 hours before coronary intervention and over the next 24 hours starting right after these procedures. A three-channel digital recorder (Aspel, HolICARD 24 W, Poland) was used and data were subsequently analyzed using a software provided by the manufacturer. Heart rate variability (HRV) was measured using lead I recording as standard deviations of normal-to-normal RR intervals (SDNN). Additionally, the mean values of 24-hour heart rate, ST-segment depression, number of ventricular and supraventricular extrasystoles, and PR, QT, QRS intervals, and QT dispersion, were analyzed. These measurements were contrasted using 1 hour periods immediately before and after the coronary procedure. Five minutes epochs of heart rhythm recordings were decomposed using the fast Fourier transformation of the data in which extrasystolic events were replaced by interpolation. The standard intervals were used for high (HF, above 0.4 Hz), low (LF, 0.4-0.15 Hz), very low (VLF, 0.04-0.015 Hz) and ultra low (ULF, 0.0033–0.04 Hz) component frequencies of the variability. High and low frequencies components were also expressed in normalized units, as a percentage of total variability. These measurements were subsequently analyzed using a software provided by the manufacturer. Heart rate variability (HRV) was measured using lead I recording as standard deviations of normal-to-normal RR intervals (SDNN).

**Measurement of urinary excretion of leukotriene E4 (uLTE4)** using high performance liquid chromatography - mass spectrometry

Urine samples were stored at -80°C. Internal standard d4-(8, 9, 10)-LTE4, and the immunoaffinity resin (cysteinyl-leukotriene affinity sorbent, Cayman Chemical, MI USA) were used for extraction of uLTE4. During the analysis uLTE4 was eluted from the reverse-phase column (C18 PepMap 100) using gradient of acetic acid and acetonitrile in water. Detection was achieved by positive ions electrospray in LCQ Advantage mass spectrometer (Thermo Finnigan, USA) operating in the single ion monitoring mode. This method was highly specific for LTE4. Average precision over the range from 4 to 100 pg of LTE4/ml urine was 8.49% and measurements accuracy was linear in this range (R²=0.995). The detection threshold was 2 pg/ml urine. Concentrations of uLTE4 were expressed in picograms per milligram of creatinine.

**Statistical analysis**

Data were analyzed using StatSoft, Inc. STATISTICA software, version 8.0. Concentrations of uLTE4 were log transformed. Non-parametric data were compared using contingency tables. Parametric data were analyzed using Student t-test.

**Table 1. Baseline characteristics and treatment of the studied groups.**

<table>
<thead>
<tr>
<th></th>
<th>Zileuton (n=54)</th>
<th>Placebo (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>60 ± 7.4</td>
<td>59.1 ± 7.9</td>
</tr>
<tr>
<td>Female/Male gender</td>
<td>20 (37%)</td>
<td>16 (32.7%)</td>
</tr>
<tr>
<td></td>
<td>34 (63%)</td>
<td>33 (67.3%)</td>
</tr>
<tr>
<td>Percutaneous coronary intervention (PCI)</td>
<td>16 (29.6%)</td>
<td>11 (22.4%)</td>
</tr>
<tr>
<td>Mean values of CRP concentration [mg/l]</td>
<td>1.32</td>
<td>1.9</td>
</tr>
<tr>
<td>Mean LDL-cholesterol concentration [mmol/l]</td>
<td>2.64 ± 0.8</td>
<td>2.74 ± 1.08</td>
</tr>
<tr>
<td>Mean contrast volume [ml]</td>
<td>153.14</td>
<td>165.9</td>
</tr>
<tr>
<td>Hypertension</td>
<td>52 (96.3%)</td>
<td>42 (85.7%)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>47 (87%)</td>
<td>44 (89.8%)</td>
</tr>
<tr>
<td>History of myocardial infarction</td>
<td>12 (22.2%)</td>
<td>12 (24.5%)</td>
</tr>
<tr>
<td>History of stroke</td>
<td>2 (3%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Peripheral artery occlusive disease</td>
<td>5 (9.3%)</td>
<td>3 (6.1%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>11 (20.4%)</td>
<td>9 (18.4%)</td>
</tr>
<tr>
<td>Renal failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Liver failure</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>History of smoking</td>
<td>35 (64.8%)</td>
<td>31 (63.3%)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>54 (100%)</td>
<td>49 (100%)</td>
</tr>
<tr>
<td>Clopidogrel or ticlopidine</td>
<td>54 (100%)</td>
<td>49 (100%)</td>
</tr>
<tr>
<td>Angiotensin converting enzyme inhibitors</td>
<td>53 (98%)</td>
<td>49 (100%)</td>
</tr>
<tr>
<td>Beta - blockers</td>
<td>52 (96.3%)</td>
<td>48 (98%)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>10 (18.5%)</td>
<td>7 (14.3%)</td>
</tr>
<tr>
<td>Nitrates</td>
<td>13 (24.1%)</td>
<td>12 (24.5%)</td>
</tr>
<tr>
<td>Statins</td>
<td>54 (100%)</td>
<td>48 (98%)</td>
</tr>
</tbody>
</table>

No significant differences in characteristics parameters were found between the study groups.

**Table 2. Angiographic characteristics of the study groups.**

<table>
<thead>
<tr>
<th></th>
<th>Zileuton (n=54)</th>
<th>Placebo (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-vessel coronary disease</td>
<td>29 (53.7%)</td>
<td>22 (44.9%)</td>
</tr>
<tr>
<td>1-vessel coronary disease</td>
<td>16 (29.6%)</td>
<td>10 (20.4%)</td>
</tr>
<tr>
<td>2-vessel coronary disease</td>
<td>5 (9.3%)</td>
<td>6 (12.25%)</td>
</tr>
<tr>
<td>3-vessel coronary disease</td>
<td>4 (7.4%)</td>
<td>11 (22.45%)</td>
</tr>
</tbody>
</table>

Grade of coronary artery disease ascertained by coronaryography - narrowing of internal artery diameter > 70% (Ringqvist et al., J Clin Inv 1983;71:1854-1866). No significant differences in characteristics parameters were found between the study groups.
multiple measurements ANOVA and post hoc hypothesis testing. This analysis tested for effects of the type of coronary procedure, time and 5-LO inhibition on changes of uLTE4 or the heart rhythm parameters. Type I error less than 0.05 was assumed statistically significant.

RESULTS

Out of 103 subjects who underwent coronary catheterization, 16 subjects in the zileuton group and 11 in the placebo group had angioplasty. Three subjects in the zileuton group and 11 in the placebo group were referred to the coronary artery bypass grafting. In 38 patients from the zileuton group and 38 patients from the placebo group coronary angiography was the only procedure performed during the study. Angiography findings are summarized in Table 2. No patient had any major acute cardiovascular events during the study period.

On the recruitment day median values of urinary LTE4 did not differ between the study groups (Table 3). Pretreatment with zileuton decreased excretion of uLTE4 by 26.02%, while it remained unchanged in the placebo group. Coronary angiography or angioplasty increased uLTE4 significantly in all subjects by 200.9%. Type of coronary procedure or zileuton treatment had no significant effect on this increase. There was no correlation between increase of uLTE4 and severity of coronary artery disease or the volume of the radiological contrast used. Elevated uLTE4 returned to the baseline levels on the next day.

Table 3. Effects of coronary intervention and zileuton treatment on urinary excretion of LTE4.

<table>
<thead>
<tr>
<th></th>
<th>Zileuton group (n=54)</th>
<th>Placebo group (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hours before PCI (recruitment)</td>
<td>86.46 [26.17 – 187.58]</td>
<td>91.27 [30.35 – 162.63]</td>
</tr>
<tr>
<td>2 hours after PCI</td>
<td>131.47 [29.22 – 375.92]</td>
<td>161.84 [60.0 – 330.49]</td>
</tr>
<tr>
<td>24 hours after PCI</td>
<td>75.83 [17.35 – 239.94]</td>
<td>85.57 [26.18 – 167.39]</td>
</tr>
</tbody>
</table>

* - urinary LTE4 concentration in pg/mg creatinine, median [5th – 95th percentile], repeated measurements ANOVA post hoc comparison of log transformed data, Newman-Keuls test: * - p<0.0001 - treatment effect, within subjects comparison to the previous urine sample, - p<0.01, # - p<0.05 - zileuton effect, between the subjects comparison of the same time urine sample,

Table 4. The heart rate before and after coronary intervention

<table>
<thead>
<tr>
<th></th>
<th>Average heart rate (bpm±SD)</th>
<th>during 24 hours before PCI</th>
<th>during one hour before PCI</th>
<th>during one hour after PCI</th>
<th>during 24 hours after PCI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zileuton (n=54)</td>
<td>63.26±8.11#</td>
<td>65.91±9.25#</td>
<td>64.42±7.52#</td>
<td>64.79±8.24#</td>
<td></td>
</tr>
<tr>
<td>Placebo (n=49)</td>
<td>68.08±10.13</td>
<td>71.16±14.81</td>
<td>69.12±12.56</td>
<td>67.67±10.91</td>
<td></td>
</tr>
</tbody>
</table>

repeated measurements ANOVA post hoc comparison, Newman-Keuls test: # - p<0.01, * - p<0.05 bpm – beats per minute

PCI – percutaneous coronary intervention
SD – standard deviation

There was a decrease in HRV following the coronary procedure (from 123.23±33.9 to 115.78±37.3 ms, ANOVA, p=0.02) in both groups, but HRV remained higher in the zileuton group by 11.2% before and 9.0% after the procedure (p=0.075). Decreases affected standard deviation of NN intervals (SDNN) and their 5 min segments (SDANN), but did not affect index of SDANN or the fraction of variant NN intervals (p50NN). Effect of zileuton was significant for SDNN (p=0.03) and for SDANN (p=0.05). Using post hoc analysis, the highest SDANN was in the zileuton group before coronary angiography (64.0±21.6 ms, p=0.026), but it decreased thereafter to the value observed in the placebo subjects (~50±24 ms). Zileuton affected mostly very low and ultra low frequencies of HRV. VLF component was higher by 33.2% before and by 26.7% after coronary intervention (p=0.016). ULF component was higher by 39.2% and by 17.7%, respectively (p=0.035; Fig. 1). Zileuton prevented decrease of normalized HF component, which did not change after the coronary procedure (51.8%±7.4 and 51.5%±9.3) in this group, but decreased from 56.8%±10.1 to 53.7%±9.6 in the placebo group (p=0.026). As expected, the opposite change was observed in normalized LF component, which increased from 43.2%±9.6 to 46.3±10.1 after the coronary procedure in the placebo group but remained unchanged in the zileuton group (p=0.019; 48.1%±8.5 and 48.5±9.8). Calculated high-to-low frequencies ratio dropped in the placebo group by 12%, but it increased by 3.4% in the zileuton group (p=0.024). No differences were found between the subjects who had angioplasty or coronary angiography only (data not shown).

There was an interesting interaction between zileuton and the coronary procedure on PQ interval. In the placebo group PQ interval was stable between 1st and 24th hour of pre-procedure recording (157.1±26.2 and 158.8±29.3 msec), but raised transiently by 15.2% in the first hour after the coronary procedure and returned to the baseline in the last 24 hour (181.0±31.7 and 161.8±25.7). This prolongation of PQ interval was totally absent in the zileuton group, in which respective PQ intervals were 164.4±24.5, 162.8±25.3, 162.5±23.6 and 161.0±22.3 msec (Fig. 2A). Analysis of variance demonstrated a highly significant effect of the coronary procedure (p<0.001), and its interaction with zileuton (p<0.001), on PQ intervals. A moderate prolongation of QRS was observed within the first hour following the procedure in all subjects (p<0.01), but it was not affected by zileuton (Fig. 2B). QT segment time changed in parallel to PQ interval (Fig. 2C). Heart rate corrected QT in the placebo group increased by 5.6% following coronary angiography (from 434.9±42.4 to 459.1±41.2 msec, but it remained unchanged in the zileuton group (445.8±36.2 and 441.2±49.9 msec). Zileuton treatment tended to prevent QT prolongation following coronary procedure (p=0.07), thus elongation of QT segment was absent in the zileuton group, while it significantly increased in the placebo group (p=0.001; post hoc). Diversity index of QT was not affected by the
The coronary procedure significantly increased number of ventricular, but not supraventricular extrasystoles (p<0.01). Zileuton had no effect on the arrhythmia episodes.

**DISCUSSION**

We sought possible effects of LTs on the heart rhythm during coronary angiography, which in 25% subjects was followed by angioplasty. The procedure is accompanied by release of CysLTs into coronary circulation (23) and subsequently into urine (24). We did not observe any differences in uLTE4 or the heart rhythm parameters, when coronary angiography was contrasted to angiography plus angioplasty. Administration of a specific 5-LO inhibitor at the time of the procedure let us observe biological effects of leukotrienes on the heart rhythm. The doubled-blind, randomized design of the study permitted for objective evaluation of the data. Assessment of urinary LTE4 was carried out by a highly specific method of quantification, which was controlled with an internal standard of deuterated LTE4. After 24 hrs 5-LO inhibitor significantly decreased urinary LTE4 excretion nearly by 26%. A similar effect was seen by us previously in asthmatic subjects (12), using the same drug and dosage.

ECG alterations can be elicited by CysLTs, as reported using animal models (16, 27-29) and are attributed to cardiac ischemia caused by a decrease in coronary perfusion. In these experiments inhibition of LTs biosynthesis protected against ischemic or reperfusion injury. In our study we used a specific inhibitor of leukotriene biosynthesis and observed its beneficial effects on the heart rhythm. It consisted in a significant fall of heart rate and an increase of heart rate variability. Moreover, inhibition of leukotrienes biosynthesis protected against a transient prolongation of atrioventricular conduction time, which accompanied coronary procedures. Zileuton decreased coronary procedure or zileuton (Fig. 2D). No zileuton effects on number or a cumulative time of ST segment depressions were noted.

The coronary procedure significantly increased number of ventricular, but not supraventricular extrasystoles (p<0.01). Zileuton had no effect on the arrhythmia episodes.

**FIG. 1.** Heart rate variability one hour before and after the coronary procedure. Panel A: very low frequency (VLF) and ultra low frequency (ULF). Panel B: normalized high frequency (HF) and low frequencies (LF). Circles – the zileuton group, squares - the placebo group. * - p<0.05.

**FIG. 2.** Comparison of standard lead 1 ECG: one hour before and after the coronary procedure. Panel A: PQ interval. Panel B: QRS complex time. Panel C: corrected QT segment time. Panel D: diversity of QT segment time. Circles – the zileuton group, squares - the placebo group. * - p<0.05.
leukotrienes biosynthesis significantly, but it did not abolish it completely. A temporary rise of uLTE4 following the procedure could be still noticed. A cellular source of CysLTs is unknown, an interaction between neutrophiles, expressing 5-LO and platelets having leukotriene C_4 synthase as an orphan enzyme, could produce CysLTs by a transcellular mechanism.

Although LTB_4 is produced at the same time, published data (30) suggest that CysLTs rather than LTB_4, are mediators of heart injury. Zileuton is the only inhibitor of 5-LO approved for use in humans. Because the drug has a rather poor pharmacokinetics, it requires a high and frequent dosage. Adverse effects of zileuton are mild and unlikely to appear during its short term application. Because the second phase metabolism of the drug shares a common pathway of cytochrome P450 with other xenobiotics, it potentially creates problems of drug interaction with digoxin, warfarin, beta-blockers or antihistamincs (31). Either a 5-LO inhibitor with improved pharmacokinetics or a specific CysLTs receptor antagonist would be more useful in prevention of cardiac conduction system alterations during ischemia and/or reperfusion. CysLTs stimulate different receptors. Two of them, CysLT_1R and CysLT_2R, are quite abundant in various tissues. CysLT_1R mediates bronchial contraction and mucosal oedema - hallmarks of asthma, while CysLT_2R causes dilatation of systemic blood vessels (25, 32). CysLT_1R was also detected on Purkinje fibers of the heart, however, its role in the conduction system is unclear. Recently a third receptor for CysLTs - GPR17 was cloned (33), also present in the heart. This receptor is stimulated also with uracil nucleotides. Specific antagonists of CysLT_1R and CysLT_2R are used for management of asthma, but they are not active in cardiovascular system. Recently, a novel mechanism dependent on CysLT_1R activation on erythrocytes was describe, which has a direct influence of CysLTs on the sinoatrial node, and acceleration of heart rate and increased heart rate variability (34). We could not control in our study for this effect since the protocol was completed before this discovery. Of the two others, CysLT_2R was found recently to be involved in ischemia and reperfusion injury of the heart (35).

Our observations of significant changes in heart rate and its variability, even before coronary procedures, shed some new light on the role of CysLTs. Beneficial response to incomplete inhibition of CysLTs biosynthesis leaves some room for more spectacular effects of more potent drugs. Over the last decades HRV turned out to be a predictive factor of cardiac death related to myocardial infarction and diabetic angioopathy (36). Despite our limited knowledge on physiological background of HRV, not only high and low frequency components, but also very low frequencies oscillations of RR interval can reflect autonomic innervations of the heart (37). Suppressed heart rate variability, especially within its high frequency component, was found predictive for myocardial infarction or unstable coronary artery disease (38). Zileuton prevented unfavorable change in high-to-low frequencies ratio of HRV, triggered transiently by the coronary procedure. The initial high-to-low ratio in the zileuton group suggests a direct influence of CysLTs on the sinoatrial pacemaker cells, rather than modulation of autonomic innervation activity. This speculation is supported by the protective effect of zileuton on ventricular repolarization, likewise pacemaker activity depending on potassium currents. Cardiac inwardly rectifying potassium channels, gated by G-protein βγ-subunits are also activated by LTs (39, 40). Alternatively, inhibition of 5-LO by zileuton could result in diminished parasympathetic stimulation of the heart mediated by cardiac muscarinic M_3 receptor, and acceleration of heart rate. Since our experimental data are in contrast with this conjecture, a direct coupling of CysLTs receptors and excitability of cardiac conduction system is more probable. In summary, we report on the effects of 5-LO inhibition in patients undergoing coronary angiography or angioplasty.

Inhibition of 5-LO pathway: 1) decreased heart rate by 4.7–7.3%, 2) increased total HRV, and specifically some of its frequency components, 3) protected against transient prolongation of heart depolarization and repolarization phases, 4) had no effect on intraventricular propagation of excitation, arrhythmias or myocardial ischemia.

Acknowledgements: This work was supported by the Polish State Research Grant 0634/P05B/2005/28 and by Polpharma Foundation for Development of Polish Pharmacy and Medicine.

Conflict of interest: None declared.

REFERENCES


Received: June 26, 2009
Accepted: January 12, 2010

Author’s address: Prof. Andrzej Szczeklik MD, PhD, Department of Medicine, Jagiellonian University Medical College, 8 Skawinska Street, 31-066 Cracow, Poland; Tel.: +48 12 430 5169, fax: +48 12 430 5115. E-mail: mmszczek@cyf-kr.edu.pl