Increased Urinary Leukotriene Excretion in Patients With Cardiac Ischemia
In Vivo Evidence for 5-Lipoxygenase Activation

Melissa Carry, MD; Victoria Korley, BSc; James T. Willerson, MD; Lynette Weigelt, RN; Anthony W. Ford-Hutchinson, PhD; and Philip Tagari, MA

Background. Experimental cardiac ischemia in some animal models results in the activation of the enzyme 5-lipoxygenase and the subsequent production of leukotrienes, potent proinflammatory lipid mediators, by the affected myocardium. Furthermore, prototype antileukotriene drugs can show some beneficial effects on infarct size and cardiac function in these models. Accordingly, urinary excretion of leukotriene E4 (LTE4), the major urinary metabolite of peptide leukotrienes in humans, was measured in patients admitted to the hospital with evidence of acute myocardial ischemia to assess in vivo release of 5-lipoxygenase products during and after the ischemic episode.

Methods and Results. Urinary leukotriene excretion was measured by reversed-phase high-performance liquid chromatography and specific radioimmunoassay on admission with acute chest pain and again on day 3 in the following patient groups: acute myocardial infarction (AMI), AMI and clinical evidence of early reperfusion after treatment with recombinant tissue-type plasminogen activator (rt-PA), diagnosis of unstable angina (UA) based on clinical history and coronary arteriography, and age-matched controls and normal hospital employees. In 16 patients with diagnosis of AMI, LTE4 excretion on admission (331±99 pg/mg creatinine sulfate; mean±SEM) was considerably higher than that measured on day 3 (195±59 pg/mg creatinine sulfate). In a subgroup of seven subjects treated with rt-PA resulting in early reperfusion, day 1 excretion was similar (215±50 pg/mg) but had significantly declined by day 3 (65±16 pg/mg; p<0.01). Urinary LTE4 excretion at admission for chest pain was also elevated in 14 patients having unstable angina (UA; 370±125 pg LTE4/mg creatinine sulfate). This had declined significantly (p<0.05) by day 3 (at which time chest pain had resolved) to 94±31 pg/mg creatinine sulfate, an excretion rate comparable with that measured in eight similarly aged subjects (64±12 pg/mg creatinine).

Conclusions. This study suggests that peptide leukotrienes are released during episodes of myocardial ischemia and provides clinical evidence for involvement of their biosynthetic enzyme, 5-lipoxygenase, during and after acute myocardial infarction and unstable angina attacks. Thus, potent and specific orally active leukotriene biosynthesis inhibitors may have therapeutic potential in limiting myocardial damage and functional abnormalities after acute ischemia. (Circulation 1992;85:230-236)

The pathogenesis of biochemical and structural changes occurring in the heart after ischemic episodes has been the subject of much study, but remains controversial. In particular, experimentally induced cardiac ischemia and reperfusion in animals has suggested a role for leukotrienes, potent inflammatory mediators generated by the action of 5-lipoxygenase,1 in both myocardial neutrophil infiltration2,3 and associated cardiac dysfunction.4,5 Prototype inhibitors of 5-lipoxygenase (5-LO) and peptidelukotriene receptor antagonists are effective in reducing infarct size and reperfusion-induced ar-

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rhythms in some animal models of experimental ischemia.6–9 Furthermore, considerably increased concentrations of 5-LO products have been detected in experimentally ischemic myocardium.10–14

Because of their rapid metabolism and excretion, leukotrienes are difficult to measure accurately in blood,15,16 although elevated plasma concentrations of these mediators have been reported after acute myocardial infarction.17,18 Such measurements are additionally complicated by the possibility of leukotriene release from eosinophilic and neutrophilic leukocytes during blood sampling and processing. Thus, the extent of their in vivo generation in clinical myocardial ischemia has remained obscure.

We have recently developed sensitive methodology to measure leukotriene E4 (LTE4), a major metabolite of peptide leukotrienes in humans,19,20 in urine from patients.21,22 Accordingly, we applied these techniques to the assessment of leukotriene excretion (as an index of the products of 5-lipoxygenase) in patients with acute myocardial ischemia to further clarify the role of leukotrienes in clinically relevant situations.

Methods

Acute Myocardial Infarction

Sixteen patients (two women and 14 men) admitted to Parkland Memorial Hospital in Dallas, Texas, with acute chest pain and myocardial infarction (AMI) were studied. Two subjects had inferoposterior infarcts, two had subendocardial (non-Q wave) infarcts, three had inferior wall infarcts, and one had an anterior wall infarct. Eight of these subjects received recombinant tissue-type plasminogen activator (rt-PA) within 4 hours of onset of chest pain, in accordance with the Thrombolysis in Myocardial Infarction Trial protocol.23 In seven patients, early reperfusion was suggested by appearance of an early (<12 hours) creatine kinase enzyme peaking with or without rapid resolution of pain and/or electrocardiographic evidence of reperfusion arrhythmias. Fourteen of the 16 AMI patients were cigarette smokers, and one was diagnosed with acute cocaine intoxication. Three subjects had coronary artery bypass surgery subsequent to the study. One patient died on the third hospital day from cardiogenic shock. Further clinical details are given in Tables 1 and 2.

Unstable Angina

Fourteen other patients (11 women and three men) admitted to the above medical center with acute chest pain were diagnosed with unstable angina (UA). Diagnosis was based on a history of UA and presence of indicative electrocardiographic changes. The subjects had clinical evidence of coronary artery disease, including previous AMI (four subjects) and left heart cardiac catheterization (13 subjects). In these patients, coronary arteriography revealed one-, two-, or three-vessel disease by the presence of ≥70% luminal coronary artery stenosis. In one subject who was not catheterized, coronary artery disease was indicated by a positive dipyridamole thallium scan. Nine of the 14 UA patients were cigarette smokers. Three subjects had coronary artery bypass graft surgery subsequent to the study, and two patients experienced recurrent symptoms requiring revascularization by angioplasty. Further details are presented in Tables 1 and 2.

Controls

Ten staff members (five men and five women) of Parkland Memorial Hospital without evidence of coronary artery disease formed a reference population. One subject was a diet-controlled diabetic and one was taking estrogen replacement therapy. Two other control groups were studied. Five healthy middle-aged men and two middle-aged men and one woman with nonischemic cardiac abnormalities (ventricular tachycardia, valvular heart disease, heart block) provided an older control set. A second control group consisted of seven subjects presenting with nonischemic chest pain (valvular heart disease, dilated cardiomyopathy, atypi-

<table>
<thead>
<tr>
<th>TABLE 1. Patient and Control Profiles</th>
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<tbody>
<tr>
<td>Acute myocardial infarction (n=16)</td>
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<tr>
<td>Received recombinant tissue plasminogen activator (rt-PA)*</td>
</tr>
<tr>
<td>Clinical evidence suggestive of early reperfusion†</td>
</tr>
<tr>
<td>Cigarette smoker</td>
</tr>
<tr>
<td>Diabetes</td>
</tr>
<tr>
<td>Male sex</td>
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<tr>
<td>Age (years, mean±SEM)</td>
</tr>
<tr>
<td>Time to first urine sample (hours, mean±SEM)</td>
</tr>
</tbody>
</table>

NA, not applicable. *See “Methods.” †Early serum creatine kinase enzyme peaking, resolution of chest pain, reperfusion arrhythmias.

<table>
<thead>
<tr>
<th>TABLE 2. Pharmacological Profile of Patients and Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute myocardial infarction (n=16)</td>
</tr>
<tr>
<td>Aspirin</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory</td>
</tr>
<tr>
<td>Nitrates</td>
</tr>
<tr>
<td>Oral hypoglycemic</td>
</tr>
<tr>
<td>Insulin</td>
</tr>
<tr>
<td>Ca2+ channel antagonist</td>
</tr>
<tr>
<td>β-Blocker</td>
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<tr>
<td>Estrogen replacement</td>
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<td>Methylprednisolone</td>
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cal chest pain, heart block) who underwent investiga-
tional cardiac catheterization. Additional information
is shown in Tables 1 and 2.

Urinary Samples

A urine sample was obtained on admission to the
hospital in 15 of 16 AMI patients at 8±2
(mean±SEM) hours after the onset of chest pain but
before cardiac catheterization (if performed). A sec-
ond sample was obtained 3 days after admission in 14
of the 16 patients. In two of the AMI patients, a
single sample was obtained 7–11 days after infarc-
tion. Samples were similarly obtained from 12 of the
14 UA patients at admission (7±2 hours) and from
12 after 3 days. Individual samples were also ob-
tained from four of these patients between 3 and 11
days after onset of chest pain. Samples were also
obtained before (two cases) and within 24 hours after
cardiac catheterization (seven cases) from patients
with nonischemic cardiac abnormalities. Urine was
stabilized by the addition of NaOH and 4-OH
TEMPO and frozen to −70°C as previously de-
scribed.21 Informed consent was obtained from all
subjects who participated in the study.

Measurement of Urinary LTE4 Excretion

Coded urine samples were analyzed by two of the
authors, blind to their origin as previously de-
scribed.21,22 Briefly, 1.14 nCi [11,12-3H]LTC4 (38.4 kCi/
mol; NEN, Lachine, Quebec) was added per millili-
ter to thawed urine samples and adjusted to pH 5.4
with acetic acid to determine analytical recoveries.
[3H]LTC4 and endogenous LTE4 were extracted from
10-ml aliquots of urine using an in-line reversed-
phase precolumn (C18 Adsorbosphere, 5-μm-diameter
packing material; Alltech, Mandel Scientific, Lach-
ine, Quebec). Leukotrienes were retrogradely eluted
via twin switching valves onto a reversed-
phase column (C18 NovaPak; Waters Co., St. Lau-
rent, Quebec) with a mobile phase consisting of
MEOH: ammonium acetate buffer (0.1% containing
1 mM disodium EDTA; pH 5.4) in the proportions
60:40 delivered at 2 ml per minute. The [3H] content
of fractions eluting with retention time of synthetic
LTC4 (see Figure 1, left peaks) was assessed by liquid
scintillation spectrometry to measure leukotriene re-
covery. Fractions eluting with the retention time of
synthetic LTE4 were evaporated to dryness under N2
gas and LTE4 concentration was measured by specific
radioimmunoassay (Figure 1, right peaks) using an
antibody demonstrating <0.1% crossreactivity with
nonleukotriene eicosanoids. The limit of detection
in this assay was 8 pg of endogenous LTE4 per milliliter
of urine. In some samples, a portion of the LTE4
immunoreactive material that coeluted with synthetic
LTE4 (Figure 2, right peak) was chemically acety-
lated by treatment with acetic anhydride in methan-
olic K2CO3. The product was rechromatographed,
revealing a peak of LTE4 immunoreactive material
that coeluted with synthetic N-acetyl LTE4 (Figure 2,
left peak). Values were normalized to the urinary

concentration of creatine sulfate and measured col-
rimetrically. Logarithmically transformed data were
analyzed for the AMI group as a whole and UA
groups separately by unpaired Student’s t test be-
cause paired samples were not available for all
subjects. Differences between subgroups of AMI
subjects were analyzed by ANOVA followed by
pooleed variance and paired t tests.

Results

Urinary LTE4 was measured in 15 samples from 16
subjects diagnosed with acute myocardial infarcti-
ion; samples were obtained 8±2 hours after commence-
ment of chest pain. Mean concentration of LTE4 in
urine was 391±101 pg/ml, giving a mean urinary
LTE4 excretion on day 1 in these subjects of 331±99
of catheterization (103±22 pg/mg; range, 12.2–179.3). The latter values were comparable with the reference population (103±42 pg LTE4/mg creatinine sulfate; range, 0.0–457.7; Figure 1). By day 3, leukotriene concentration was significantly reduced (p<0.05) to 140±21 pg/ml (excretion rate, 195±59 pg/mg creatinine; not significantly different from day 1). Values obtained from two subjects 7–11 days after infarction and reperfusion (190 and 85 pg/mg) were closer to controls. In a subgroup of seven AMI patients who received rt-PA and demonstrated evidence of early reperfusion (see “Acute Myocardial Infarction”), LTE4 excretion was significantly reduced by day 3 to 65±16 pg/mg creatinine sulfate compared with that measured in the first sample (215±50 pg/mg). This day 3 value was, however, not significantly lower than that measured in the group without evidence of reperfusion (ANOVA and pooled variance t test). Further details are given in Table 3.

The fourteen patients who were diagnosed with unstable angina had urinary LTE4 concentrations in 12 samples collected 7±2 hours after the start of symptoms of 170±43 pg/ml, giving a mean LTE4 excretion rate of 370±125 pg/mg creatinine. By day 3, chest pain had resolved in 12 of the UA patients. At this time, urinary LTE4 concentrations had significantly (p<0.05) declined to 48±13 pg/ml, with a similarly significant drop in excretion rates to 94±31 pg/mg creatinine compared with day 1. The detection of acutely increased concentrations of urinary LTE4 in a patient with UA is shown in Figure 1.

Single samples collected from days 3–11 in four subjects showed values comparable with controls (107±28 pg/mg). A single sample collected on day 2

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**TABLE 3. Urinary Leukotriene E4 in Myocardial Ischemia Subjects and Controls**

<table>
<thead>
<tr>
<th>LTE4 conc (pg/ml)</th>
<th>AMI with clinical evidence of early reperfusion</th>
<th>AMI without clinical evidence of early reperfusion</th>
<th>Unstable angina</th>
<th>Clinical controls</th>
<th>Instrumented controls</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AMI all</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>391.2±100.8</td>
<td>362.0±100.2</td>
<td>416.7±166.8</td>
<td>169.2±43.2</td>
<td>95.3±22.6</td>
<td>111.6±46.5</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Day 3</td>
<td>139.8±21.1†</td>
<td>69.3±18.8§§</td>
<td>210.3±37.3</td>
<td>48.2±12.8†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>12</td>
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</table>

<table>
<thead>
<tr>
<th>LTE4 excretion (pg/mg Cr)</th>
<th>AMI with clinical evidence of early reperfusion</th>
<th>AMI without clinical evidence of early reperfusion</th>
<th>Unstable angina</th>
<th>Clinical controls</th>
<th>Instrumented controls</th>
<th>Normal controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>330.7±98.6</td>
<td>215.1±49.9</td>
<td>431.9±171.8</td>
<td>369.8±125.3</td>
<td>63.5±11.9</td>
<td>102.5±21.9</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>12</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Day 3</td>
<td>194.5±58.5</td>
<td>65.4±15.6‡</td>
<td>298.8±90.7</td>
<td>93.9±30.8†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>12</td>
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</tbody>
</table>

AMI, acute myocardial infarction; LTE4, leukotriene E4; conc, concentration; Cr, creatinine.

*See “Acute Myocardial Infarction.”
†Significantly different from day 1 by unpaired t test (p<0.05).
‡Significantly different from day 1 by paired t test (p<0.01).
§Significantly different from day 1 by ANOVA and pooled variance t test (p<0.05).
||Significantly different from day 3 values in AMI subjects without evidence of early reperfusion by ANOVA and pooled variance t test.
from one subject with prolonged ischemia documented by continued chest pain and electrocardiogram changes and requiring urgent cardiac catheterization showed an excretion rate of 2,725.6 pg LTE4/mg creatinine, considerably greater than that seen in the catheterized, nonischemic control group (103±22 pg/mg). In two subjects from this group, precatheterization values (21.1 and 84.3 pg LTE4/mg) were comparable with postcatheterization values (12.2 and 121.9 pg LTE4/mg, respectively).

Discussion

Acute myocardial ischemia is an important cause of morbidity and mortality. The use of thrombolytic interventions in patients with thrombus-induced myocardial ischemia shows considerable success in reducing infarct size and restoring ventricular function.23,24 However, evidence is accumulating that reperfusion of ischemic heart tissue may exacerbate subsequent myocardial damage.25,26 In particular, leukocytic infiltration of the myocardium has been well documented during experimental ischemia and reperfusion.27–29 Also, drugs that nonspecifically inhibit polymorphonuclear function and compounds or antibodies that prevent their adherence to endothelium attenuate infarct damage in some experimental models.30–33

Various substances have been proposed as mediators of PMN adhesion and infiltration into ischemic myocardium. Leukotriene B4 (LTB4) is the major product of the oxidative metabolism of arachidonic acid by the enzyme 5-lipoxygenase (5-LO) in neutrophils. It is potently chemotactic for PMN, and an increased generation of LTB4 ex vivo in activated PMN from both AMI and UA patients has recently been described.34 LTB4 is rapidly metabolized to nonimmunoreactive α- and β-oxidized metabolites,35 and the possibility of its release from activated PMN during blood sampling makes reliable measurements of circulating LTB4 extremely difficult.

However, adhesion of human PMN to the vascular endothelium results in an increased synthesis of another 5-LO product, the peptide leukotriene LTC4,36 and the ability of neutrophils to export LTA4 (a common precursor of LTB4 and LTC4) to platelets and endothelial cells for conversion to peptide leukotrienes is well documented.37 Furthermore, in myocardial infarction, the infiltrating leukocytes may contain eosinophilic PMNs,38 which release large quantities of LTC4 in the presence of neutrophils.39

In humans, LTC4 is metabolized to a stable, intermediate LTE4 before β- and ω-oxidation. Thus, LTE4 is the major urinary metabolite up to 8–12 hours after administration or release of peptide leukotrienes19,20 and can be measured noninvasively and without artifacts generated in sampling.21 Accordingly, we assessed urinary LTE4 excretion as an index of 5-LO activation in a heterogeneous population of patients with myocardial ischemia in whom reperfusion might occur. We selected acute myocardial infarction patients (with or without thrombolytic therapy) and admissions with unstable angina. The latter condition is associated with intracoronary thrombus-induced ischemia and spontaneous lysis and reperfusion.40

Reversed-phase high-performance liquid chromatography and radioimmunoassay analysis showed a considerably increased concentration of LTE4 in urine sampled immediately after ischemia from a subject with unstable angina (Figure 1, upper panel) compared with urine collected 2 days subsequently. This peak could be chemically converted to its N-acetyl derivative, with a corresponding shift in retention time (Figure 2) authenticating it as LTE4. In 14 UA subjects, the concentration of LTE4 in urine sampled 7±2 hours after onset of chest pain was threefold higher (169±43 pg/ml) than that in samples taken 2 days subsequently, when pain had resolved (48±13 pg/ml, p<0.005). This significant immediate postischemia increase was preserved when values were normalized for urinary creatinine concentration (Figure 3), indicative of considerable peptide leukotriene generation (and thus, 5-LO activation) during the ischemia and spontaneous reperfusion described in this disease.40 The highest value recorded in these subjects (2,725.6 pg/mg creatinine) was observed in a single patient after 2 days of symptoms, suggesting a cumulative generation of 5-LO products.

A similar significant increase in urinary LTE4 concentration (picograms per milliliter) was observed in samples from AMI subjects collected 8±2 hours after infarct (Table 3) compared with later samples (see “Results”), indicating increased 5-LO activity during acute myocardial infarction. In the subgroup of AMI patients receiving rt-PA and showing evidence of early reperfusion, both urinary LTE4 concentrations and excretion (normalized to creatinine
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leukotriene antagonist on myocardial injury in a canine coronary occlusion-reperfusion model. Prostaglandins 1988;35:555–569


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8. Toki Y, Hieda N, Torii T, Hashimoto H, Ito T, Ogawa K, Satake T: The effects of lipooxygenase inhibitor and peptidoconcentration) were significantly reduced by day 3 compared with both day 1 reperfusion values and day 3 values from subjects without evidence of reperfusion (Table 3). This suggests that in myocardial ischemia that is persistent or in which reperfusion occurs partially and/or later, sustained generation of leukotrienes may result. This generation is probably not related to the instrumentation of these subjects (which occurred after collection of the day 1 sample) because LTE4 excretion measured within 24 hours of cardiac catheterization in subjects without ischemia was not significantly different from control groups.

The elevated LTE4 excretion rates measured shortly after the onset of chest pain in this study were similar to those in allergic asthmatic patients during significant antigen-provoked bronchoconstriction. Thus, myocardial ischemia and possible reperfusion in patients with UA and AMI may release pathophysiologically relevant concentrations of peptide leukotrienes.

These mediators and LTB4 can reproduce some of the effects of experimental ischemia and reperfusion,5,6 and 5-LO products have been detected in experimentally ischemic hearts.10–14 Inotropic and cardiovascular constrictive effects of leukotrienes have also been described.5,41 Additional evidence for production of 5-LO metabolites in myocardial ischemia comes from studies in animals with prototype 5-LO inhibitors and leukotriene antagonists. Several of these compounds showed considerable reduction in infarct size and functional improvement in various species of experimental animals,5,6,9 although not all are active.42,43

Thus, the detection of increased excretion of leukotriene metabolites in clinical acute ischemia, combined with the efficacy of several prototype antileukotriene compounds in animal models, suggests that potent and specific orally active leukotriene biosynthesis inhibitors and antagonists may have therapeutic potential in myocardial ischemia and reperfusion injury.


KEY WORDS • leukotrienes • angina • myocardial infarction
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