Transcardiac Release of Leukotriene C₄ by Neutrophils in Patients With Coronary Artery Disease

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Leukotriene C₄ is a potent constrictor of smooth muscle in vitro and may induce coronary vasoconstriction in vivo. To study leukotriene C₄ release by neutrophils in patients with coronary artery disease, neutrophils were separated from blood samples taken from the coronary sinus and aorta in 20 patients with stable exertional angina and angiographically documented coronary artery narrowings (group I). Eight patients with normal coronary arteries were also studied (group II).

To assess leukotriene C₄ generation, neutrophils were incubated with calcium ionophore A 23187 (0.25 μM) and the supernatants obtained after centrifugation were analyzed for leukotriene C₄ by radioimmunoassay.

Patients in group I had a significantly lower release of leukotriene C₄ from neutrophils separated from the coronary sinus blood than from those separated from aortic blood (4.33 ± 0.69 versus 5.92 ± 0.54 ng/ml, p < 0.025), whereas patients in group II had a similar release of leukotriene C₄ by the neutrophils separated from coronary sinus blood and from aortic blood (6.0 ± 0.72 versus 6.4 ± 0.66 ng/ml, p = NS). Moreover, in group I patients, a significant correlation was found (p < 0.01) between the extent of coronary artery disease (expressed by the Leaman coronary score) and the percent reduction in leukotriene C₄ released from neutrophils separated from coronary sinus blood as compared with leukotriene C₄ produced by neutrophils separated from aortic blood.

These data show that neutrophils from patients with coronary artery disease have a reduced ability to produce leukotriene C₄ after stimulation by calcium ionophore A 23187. The release of leukotriene C₄ from neutrophils passing through a diseased coronary tree may contribute to the development of the inappropriate vasoconstriction associated with the endothelial dysfunction of atherosclerotic coronary vessels.

Methods

Study patients. Twenty-eight patients who underwent diagnostic coronary arteriography were studied. All patients gave written informed consent and the protocol was approved by the hospital Ethics Committee. Twenty patients (group I) had a clinical diagnosis of exertional angina and a positive exercise test, defined as the development of ST segment depression ≥ 1 mm. All patients were men with a mean age of 53 years (range 30 to 67). Of the remaining eight patients (group II), seven complained of chest pain but had a negative exercise test, and one had moderate mitral valve prolapse with mitral regurgitation. There were five men and three women with a mean age of 52 years (range 42 to 63).

At the time of the study, no patient had congestive heart failure or an acute infective disease. Cardioactive drugs, which included nitrates, nifedipine and beta-adrenergic blockers in group I patients, were progressively tapered and finally discontinued ≥24 h before the study (72 h for beta-blockers). No patient was taking platelet antiaggregating agents. Significant coronary artery disease was defined as > 50% narrowing in the luminal diameter of any individual coronary vessel. For each patient, a coronary score was computed according to the method proposed by Leaman et al. (6).
Study protocol. Patients were studied in the fasting state after premedication with diazepam (10 mg). At the beginning of the procedure, a no. 8 Lehman catheter was advanced from the brachial artery and placed in the ascending aorta and a no. 7 Gorlin catheter was inserted in an antecubital vein and placed in the coronary sinus. Simultaneous sampling (15 ml of heparinized blood) was obtained from the aorta and coronary sinus before the injection of contrast medium.

Separation of neutrophils. Dextran 6% (20 ml) was added to blood samples that were immediately placed in a carbon dioxide incubator at 37°C. The polymorphonuclear leukocytes were purified by means of the Isopaque-Ficoll system (7). Contaminating erythrocytes in the neutrophil fraction were removed by lysis with 0.75% ammonium chloride solution containing Tris hydrochloride buffer (20 mM, final pH 7.4) and 0.25% autologous plasma. The purity of the cell fractions was 96 ± 2% neutrophils. Granulocytes were resuspended in sodium chloride (145 mM) containing HEPES (10 mM), potassium hydroxide (5 mM), calcium chloride (1.3 mM) and magnesium chloride (1.2 mM), pH 7.45 (HEPES buffer). Cell viability was >95% as measured by trypan blue exclusion or release of the cytoplasmic enzyme lactic dehydrogenase. Only the platelet free neutrophil suspensions were tested. Cells were evaluated within 30 min of the separation procedure.

Granulocyte leukotriene C₄ generation. To assess leukotriene C₄ generation, neutrophils (3 × 10⁶/ml) were incubated at 37°C for 15 min in a shaking water bath, with calcium ionophore A 23187 (0.25 μM), a concentration at which maximal leukotriene C₄ production occurs. The reaction was stopped by placing the tube in ice for 5 min and subsequently adding cold methanol (0.5 ml). Suspensions were centrifuged at 11,000 × g for 5 min at 4°C and supernatants were collected and stored at −70°C. Supernatants were analyzed for leukotriene C₄ levels by radioimmunoassay (New England Nuclear Chemicals).

From these values, we subtracted the levels of leukotriene C₄ obtained in a similar way in the control experiments, in which the same quantity of phosphate buffer saline solution was added to granulocyte suspensions. The selectivity of the assay is high, with low cross-reactivity with other leukotrienes (B₄ 0.03%, D₄ 11.6%, E₄ 3.3%).

Statistical analysis. All data are expressed as mean values ± SEM. Student’s t test for paired data was used to compare mean values calculated from continuous data. The correlation between the Leaman coronary score and the percent reduction in leukotriene C₄ generation in coronary sinus blood compared with aortic blood was calculated by the Spearman rank correlation coefficient.

Results

Clinical and angiographic findings. All 20 patients in group I had significant coronary artery disease, involving one vessel in 7 patients and two or more vessels in 13. Mean ejection fraction was 0.59 (range 0.38 to 0.77). All eight patients in group II had normal coronary arteries and the mean ejection fraction was 0.58 (range 0.52 to 0.80).

Leukotriene C₄ generation by stimulated neutrophils (Fig. 1 and 2). Mean leukocyte count was 7.56 ± 0.84 in group I and 7.13 ± 1.12 in group II (p = NS). Group I patients had a significantly lower release of leukotriene C₄ from neutrophils separated from coronary sinus blood than from those separated from aortic blood (4.33 ± 0.69 versus 5.92 ± 0.54 ng/ml, p < 0.025). Group II patients had a similar release of leukotriene C₄ by neutrophils separated from coronary sinus blood and from aortic blood (6.0 ± 0.72 versus 6.4 ± 0.66 ng/ml, p = NS) (Fig. 1). In group I patients, a significant correlation was found between the Leaman coronary score and the percent reduction in leukotriene C₄ release from neutrophils separated from coronary sinus blood compared with leukotriene C₄ release from neutrophils separated from aortic blood (r = 0.71, p < 0.01) (Fig. 2).

Figure 1. Mean values ± SE of leukotriene C₄ (LTC₄) release by stimulated neutrophils separated from blood samples taken from the coronary sinus and aorta in 20 patients (PTS) with coronary artery disease (CAD) and 8 control subjects with normal coronary arteries.

Figure 2. In the 20 patients with coronary artery disease, a significant linear correlation was found between the Leaman coronary score and the percent reduction in leukotriene C₄ (LTC₄) release by stimulated neutrophils separated from coronary sinus blood compared with aortic blood.
Discussion

Biosynthesis and effects of leukotriene C₄. Arachidonic acid, a component of the membrane phospholipids of mammalian cells, is rapidly metabolized to a variety of compounds, depending on the enzyme present in the tissue. In platelets, arachidonic acid is metabolized to thromboxane A₂ and prostaglandins by the cyclooxygenase pathway, whereas in leukocytes, arachidonic acid is preferentially metabolized to leukotrienes through the lipoxygenase pathway (7-9). Leukotriene C₄ and its metabolites leukotriene D₄ and leukotriene E₄ were originally described as “slow-reacting substances of anaphylaxis” (10) and are now collectively referred to as “peptide leukotrienes” (11). The generation and release of such compounds have been demonstrated in human neutrophils, eosinophils, mast cells, macrophages and monocytes. Under certain conditions, endothelial cells and smooth muscle cells may also synthesize leukotriene C₄ by conversion of exogenous leukotriene A₄ (12).

Peptide leukotrienes are very potent “proinflammatory” mediators that are able to contract smooth muscle cells from bronchus and gastrointestinal tissues, induce vasoconstriction and increase endothelial cell permeability (3). Vasoconstriction has been demonstrated by the ability of leukotriene C₄ and leukotriene D₄ to increase systemic vascular resistance in the rat and diminish coronary blood flow in the isolated guinea pig heart (13). Ezra et al. (5) injected increasing doses of leukotriene C₄, D₄ and E₄ into the left anterior descending artery of eight open chest domestic pigs. A significant dose-related reduction in coronary blood flow was observed, frequently accompanied by electrocardiographic signs of myocardial ischemia and an increase in left ventricular filling pressure. Recently, Marone et al. (11) studied the effects of the intravenous injection of a small dose (3 nmol) of leukotriene D₄ in patients with normal coronary arteries. The authors found a progressive increase in coronary vascular resistance that peaked 15 min after injection with return to baseline values at 20 min. Such increase was associated with greater myocardial oxygen extraction, whereas myocardial oxygen consumption did not change.

The vasoconstrictor effect of leukotriene C₄ on coronary arteries is synergistic with that induced by platelet-released thromboxane A₂ because experimental data (8) show that the decrease in coronary blood flow produced by the combination of both substances is greater than the sum of changes caused by the two eicosanoids administered separately. Although the recognition of these properties led to the hypothesis that leukotrienes could be implicated in vaso spas tic disorders, a role for these substances in the pathogenesis of myocardial ischemia has never been demonstrated. One main difficulty is related to the short half-life of leukotrienes in the blood circulation, thus precluding meaningful determinations in blood plasma under most conditions (14,15).

Transcardiac generation of leukotriene C₄ by stimulated neutrophils. In this study, neutrophils were separated from blood samples taken from patients with coronary artery disease and subjects with normal coronary arteries. After stimulation with calcium ionophore A 23187, supernatants were analyzed for leukotriene C₄ by radioimmunoassay. In each patient, two measurements were obtained, one after stimulation of neutrophils separated from blood samples taken from the aorta and the other after stimulation of neutrophils collected from the coronary sinus, to assess whether the passage through the coronary circulation would modify the response of leukocytes to the same stimulus. Our results show that in patients with coronary artery disease, neutrophils separated from coronary sinus blood released a smaller amount of leukotriene C₄ than did neutrophils isolated from aortic blood, whereas no difference was found in the subjects with normal coronary vessels. Moreover, a significant correlation was found between the extent of coronary artery disease and the percent reduction in leukotriene C₄ generation by neutrophils isolated from coronary sinus compared with aortic blood. One explanation is that neutrophils can be activated during their passage over the atheromatous plaques of coronary arteries and that they generate leukotriene C₄. Prior activation in the coronary arteries may account for the lower release of leukotriene C₄ by neutrophils separated from the coronary sinus blood of patients with coronary artery disease. Recent data (1) suggest that neutrophils have increased activity in patients with ischemic heart disease. The basis for such enhanced function may relate to complement activation because the terminal C₅b-9 complement complex has been localized in coronary fibrous plaques, suggesting complement activation in atherosclerotic arterial tissues (16).

Alternative explanation for the reduced release of leukotriene C₄ by neutrophils collected from coronary sinus blood from patients with coronary artery disease. Neutrophils may transfer leukotriene A₄, the immediate precursor of leukotriene C₄, to endothelial cells. There is considerable evidence (12) that vascular cells are unable to generate leukotriene A₄ (and hence leukotriene C₄) from arachidonic acid, but they are able to synthesize leukotriene C₄ from exogenous sources of leukotriene A₄. Although endothelial and smooth muscle cells may be important contributors to the total production of leukotriene C₄ (17,18), they must interact with other cell types, such as neutrophils, that serve as a source of leukotriene A₄ (12). It is known that injured endothelium promotes neutrophil adhesion to the vessel wall (19) and exposure of endothelial receptors for C₅b has been reported (20) after endothelial injury. These receptors could act to localize complement to the vessel wall and thereby increase neutrophil adherence through similar receptors present on the neutrophils. As a consequence of the close interactions between damaged endothelium and neutrophils, these leukocytes could transfer the metabolic precursor leukotriene A₄ to the vascular cells. As a result, neutrophils separated from coronary sinus blood of patients with athero-
sclerotic involvement of coronary vessels would release smaller quantities of leukotriene C$_4$ after stimulation by calcium ionophore.

**Implications.** Our data show a reduced ability of neutrophils after stimulation by calcium ionophore to release leukotriene C$_4$ once they have passed through a diseased coronary tree. In contrast to endothelium-dependent relaxation, endothelium-dependent contraction becomes prominent under certain conditions, including atherosclerosis, favoring the occurrence of vasoconstriction (21). Chronic endothelial injury and coronary stenosis lead to the accumulation of platelets and white blood cells that can release potent vasoconstrictor mediators (22). Leukotriene C$_4$ production by neutrophils may contribute to the development of the inappropriate vasoconstriction that occurs as a result of the endothelial dysfunction associated with the atherosclerotic involvement of coronary vessels.

**References**