EFFECTS OF LEUKOTRIENE D₄ ON MYOCARDIAL BLOOD
FLOW AND HIGH ENERGY PHOSPHATE CONCENTRATION
IN ANESTHETIZED DOGS

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The effects of intracoronary administration of leukotriene D₄ (LTD₄) on myocardial blood flow (MBF) and myocardial energy metabolism in anesthetized open-chest dogs were examined, and compared with those of coronary ligation. Two series of experiments were conducted. In the first, LTD₄ (0–3.0 µg/kg) was injected into the left anterior descending coronary artery (LAD) and MBF was measured. While no changes in MBF were observed after 0.5 µg/kg of LTD₄, a significant decrease in MBF in the LAD area was apparent after 1.0 µg/kg of LTD₄, with a return to baseline values by within 10 min after the injection. With 3.0 µg/kg of LTD₄, MBF remained decreased up to 15 min after the injection. In the second study, myocardial high energy phosphate concentrations in the LAD area were determined 5 min after LTD₄ administration and compared to those after ligation. ATP levels in the 1.0–3.0 µg/kg LTD₄ groups were significantly less than those in the ligation group, although there were no associated significant differences in MBF values in the LAD area. These results indicate that LTD₄ brings about changes in myocardial energy metabolism which are not secondary to reduced blood flow.

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Acute anaphylaxis is characterized by severe hemodynamic changes, cardiac arrhythmias and myocardial ischemia. All of these phenomena can be elicited by systemic or intracardiac administration of slow-reacting substances of anaphylaxis, which were originally demonstrated to be products of leukocytes but have since been shown to be produced by a variety of tissues, including the coronary and pulmonary arteries. The peptide-containing leukotrienes, i.e., leukotriene C₄ (LTC₄), leukotriene D₄ (LTD₄), and leukotriene E₄, are known to possess potent biological properties including bronchoconstriction, increased vascular permeability and vasoconstriction.

Recently, LTC₄ and LTD₄ have been identified as the major active constituents of slow-reacting substances of anaphylaxis and shown to be potent constrictors of coronary arteries. In addition, peptide leukotrienes are known to depress cardiac function, produce vasoconstriction, increase vascular permeability and bring about coronary blood flow imbalance. However, little attention has been directed toward the effect of LTD₄ on myocardial energy metabolism.

We previously demonstrated using anesthetized dogs that coronary ligation ischemia-induced mitochondrial dysfunction and degradation of high energy phosphates depended on the degree of the decrease in

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- Canine heart

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myocardial blood flow (MBF) in the area concerned. The purpose of the present study was to examine MBF and hemodynamic effects of intracoronary LTD$_4$ in anesthetized, open-chest dogs. An additional objective was to assess the effects of LTD$_4$ on myocardial energy metabolism and to compare these changes with those due to coronary ligation.

**MATERIALS AND METHODS**

**Animal Preparation**

Seventy-one adult mongrel dogs of both sexes, weighing 7 to 13 kg, were used in the investigation. They were anesthetized by intraperitoneal administration of 50 mg/kg of pentobarbital, endotracheal intubation was performed, and their lungs were ventilated with room air. Aortic blood pressure was monitored through a polyethylene catheter passed retrograde from the femoral artery. The chest was opened with a fourth intercostal incision, and the heart was suspended in a pericardial cradle.

**Experimental Protocol**

Pure synthetic LTD$_4$ (Wako Pure Chemical Industries, Ltd., Osaka) was dissolved in methanol, and two series of experiments were conducted. In the first, LTD$_4$ was injected into the left anterior descending coronary artery (LAD) at doses of 0 (vehicle), 0.5, 1.0, 2.0 or 3.0 $\mu$g/kg within 5 sec. LAD ligation using a silk suture was performed on a separate group of dogs. MBF values were measured before and 5, 10 and 15 min after administration of LTD$_4$ or its vehicle or after LAD ligation, in the area of the LAD and the left circumflex coronary artery (LCX), as shown in Fig. 1.

In the second study, animals were divided into five groups: four groups were given an administration of LTD$_4$ at doses of 0, 1.0, 2.0, or 3.0 $\mu$g/kg into the LAD; the other group underwent LAD ligation. Similar levels of MBF reduction were only observed at 5 min after treatment for the four groups, except for that which received the vehicle alone, as in the first study. Therefore, myocardial specimens were taken from the LAD area where MBF values were measured 5 min after LTD$_4$ administration or LAD ligation, for determination of tissue ATP and creatine phosphate (CP).

After the administration of 2.0 or 3.0 $\mu$g/kg of LTD$_4$ and after coronary ligation, 2 of 15, 7 of 17 and 1 of 12 animals, respectively, died and these were excluded from the data analysis.

All surgical procedures and experimentation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

**MBF Measurements**

MBF was measured by polarographic recording of hydrogen desaturation with platinum electrodes as described by Aukland et al.\(^5\) Two wire-type platinum electrodes (0.08 mm in diameter) were introduced into the heart muscle and fixed in position at a depth of 5 mm from the epicardial surface (mid-myocardium); one in the LAD area, the other in the LCX area, as schematically indicated in Fig. 1. The electrodes were connected to a tissue rheometer (UH Meter PHG 201, Unique Medical Co., Ltd., Tokyo) and the hydrogen gas method was used. Inhaled gas was changed from room
TABLE 1  EFFECTS OF INTRACORONARY LTD4 ADMINISTRATION OR CORONARY LIGATION ON BLOOD PRESSURE AND HEART RATE

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>DBP (mmHg)</th>
<th>HR (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>before</td>
<td>after</td>
<td>before</td>
</tr>
<tr>
<td>Control</td>
<td>(5)</td>
<td>106.3±6.6</td>
<td>104.9±9.6</td>
</tr>
<tr>
<td>0.5 μg/kg LTD4</td>
<td>(5)</td>
<td>103.4±8.5</td>
<td>101.5±11.7</td>
</tr>
<tr>
<td>1.0 μg/kg LTD4</td>
<td>(12)</td>
<td>94.7±5.2</td>
<td>93.0±6.0</td>
</tr>
<tr>
<td>2.0 μg/kg LTD4</td>
<td>(13)</td>
<td>109.0±3.1</td>
<td>80.3±7.9*</td>
</tr>
<tr>
<td>3.0 μg/kg LTD4</td>
<td>(10)</td>
<td>112.6±6.4</td>
<td>68.4±10.1*</td>
</tr>
<tr>
<td>Ligation</td>
<td>(11)</td>
<td>107.2±5.0</td>
<td>100.2±6.6</td>
</tr>
</tbody>
</table>

LTD4, leukotriene D4; SBP, systolic blood pressure; DBP, diastolic blood pressure HR, heart rate. Blood pressure and HR were measured before and 5 min after intracoronary administration of LTD4 or its vehicle (control) or coronary ligation. Each value represents the mean±SE of data for the number of dogs indicated in parentheses.

*p<0.05 compared with the pre-treatment value.

Air to a gas mixture containing about 8% hydrogen. After 3 min, the gas was switched back to room air, and the hydrogen in the myocardium was washed out. MBF was calculated using Kety's approach to blood-tissue exchange of inert gases.

High Energy Phosphate Determinations

Five min after LTD4 administration or LAD ligation, a specimen of the cardiac muscle corresponding to the LAD area of electrode implantation was collected in about one sec, using a biopsy drill with a diameter of 3 mm and rapidly frozen in liquid nitrogen. The middle myocardial layer was used as a sample for determination of tissue ATP and CP. Each frozen myocardial tissue sample was weighed and homogenized with 2.5 ml of 7% perchloric acid per 100 mg tissue at 0°C. After deproteinization, tris buffer containing 4N-KOH was added to the homogenate to neutralize the perchloric acid, and the sample was centrifuged at 3000 rpm for 10 min. The ATP and CP contents were determined by a modification of McElroy-Strehler's firefly luminescence method using a firefly lantern extract (FLE 50, Sigma Chemical Co., St. Louis, Mo.). The measurements were made with a bioluminescence reader (Model BLR-101 C, Aloka Co., Tokyo).

Statistical Analysis

The results are presented as the mean±SE. Statistical analysis was carried out by an analysis of variance with Scheffe's test for multiple comparisons. The probability was considered significant if less than 0.05.

RESULTS

Hemodynamic Effects

Table 1 summarizes the effects of intracoronary LTD4 or coronary ligation on blood pressure and heart rate. LTD4 administration resulted in a significant decrease in systolic blood pressure as compared with pre-administration values at the higher doses (2.0 and 3.0 μg/kg). However, systolic blood pressure was unchanged with the lower LTD4 doses or coronary ligation. The only significant change in diastolic blood pressure was 5 min after treatment with 3.0 μg/kg LTD4. There were no significant changes in heart rate after any LTD4 dose or after coronary ligation.

Effects of LTD4 or Coronary Ligation on MBF

Fig. 2 shows the time courses of the effects of LTD4 at different doses or coronary ligation on MBF. In the LAD and LCX areas, MBF was unchanged in the control (vehicle only) and LTD4 0.5 μg/kg groups (Fig. 2A, B). In contrast, 5 min after injection of 1.0 μg/kg LTD4, MBF reduced in both the LAD and LCX areas, with the decrease being significantly larger in the LAD area. Ten min after LTD4 administration, the MBF values had returned to the pre-administration levels in both areas (Fig. 2C). LTD4 doses of 2.0 μg/kg (Fig. 2D) and 3.0 μg/kg
Fig. 2. Effects of intracoronary administration of leukotriene D4 (LTD4) or coronary ligation on time course changes in myocardial blood flow (MBF), measured before and 5, 10 and 15 min after treatment. Graphs show the mean±SE MBF values at the areas of the left anterior descending coronary artery (solid circles) and the left circumflex coronary artery (open circles) in vehicle (A, n=5), 0.5 μg/kg (B, n=5), 1.0 μg/kg (C, n=6), 2.0 μg/kg (D, n=7) and 3.0 μg/kg (E, n=5) of LTD4 and coronary ligation (F, n=5) groups. *p<0.05, **p<0.01

(Fig. 2E) produced a similar reduction of MBF in the LAD and LCX areas 5 min after treatment. However, at 10 and 15 min after the 3.0 μg/kg dose, MBF in the LAD area had failed to recover. A marked decrease in MBF in the LAD area occurred after coronary ligation. In clear contrast, MBF in the LCX area showed no significant change (Fig. 2F). There were no significant differences in MBF in the LAD area 5 min after treatment among the 1.0, 2.0 and 3.0 μg/kg LTD4 infusion and coronary ligation groups.

Myocardial High Energy Phosphates

There was no significant correlation between tissue contents of high energy phosphates and MBF values in the three LTD4 and ligation groups.

Fig. 3 illustrates ATP, CP and ATP plus CP contents in the LAD myocardial specimens after LTD4 administration or coronary ligation. ATP content in the ligation group (5.28±0.28 μmoles/g wet weight) was not significantly different than that in the control group, but was significantly higher than those in all three LTD4 groups. CP in the 2.0 μg/kg of LTD4 group was significantly higher than that in the ligation group value (7.19±0.28 vs 5.44±0.80 μmoles/g wet weight). CP in the other LTD4 groups also
The purpose of the present investigation was to estimate the effects of intracoronary administration of LTD₄ on MBF and high energy phosphate metabolism. Our results revealed a dose-related reduction in blood flow and deterioration of myocardial energy metabolism with no significant correlation to the MBF decrease.

Since the potential involvement of leukotriene in angina pectoris induced by coronary vasospasm has drawn much attention, mechanistic aspects have been studied extensively. The release of a LTD₄-like substance following experimental myocardial infarction in rabbits has been reported. In addition, several studies have demonstrated a dose-dependent reduction in coronary blood flow and an increase in coronary vascular resistance following injection of LTD₄. In the present study, MBF was unchanged in the LTD₄ 0.5 μg/kg group and similar levels of MBF reduction in the LAD area were observed 5 min after intracoronary injection of LTD₄ at doses of 1.0 – 3.0 μg/kg. However, 10 min after administration, MBF values remained depressed only with a high (3.0 μg/kg) dose of LTD₄. Our findings showed a dose-related decrease in tissue blood flow (MBF), which agrees with the results of earlier reports concerning coronary blood flow.

Moreover, a significant decrease in MBF in the LCX area was apparent 5 min after LAD administration of LTD₄. Torii et al described using open-chest anesthetized dogs that 1 min after injection of a 3.0 μg (approximately 0.2 μg/kg) dose of LTD₄ into the LAD, MBF in the LAD area and the adjacent area was reduced significantly. Several articles have reported that intracoronary infusion of LTD₄ reduces cardiac contractility in experimental animals, possibly reflecting negative inotropic effects. Although cardiac function was not determined in our investigation, the possibility that negative inotropic effects of LTD₄ reduce MBF in the LCX area requires consideration.

LTD₄ is rapidly inactivated in blood, which makes it difficult to measure its formation in vivo. Therefore, little attention has been directed toward quantitating the
amount of blood LTD₄ induced by acute myocardial ischemia. LTD₄ doses of 0.01
- 5 μg/kg were used in earlier studies to test the effects of intracoronary administration in
anesthetized open-chest dogs²⁸,²⁴,²⁵ Thus, the doses used in this study were consistent with
those in previous reports.

Cardiac performance is directly related to tissue ATP concentrations. Myocardial
ischemia perturbs high energy phosphate metabolism in cardiac myocytes, which leads
to a decline in contractile function²⁸-³⁰ ATP content in myocardium thus appears to
reflect cellular viability³⁰,³¹ and it has been proposed that ATP levels depend largely
on the degree of MBF²³,³¹ In our investigation, therefore, the area at which MBF was mea-
sured corresponded with the location where tissue samples were taken for determination
of high energy phosphate contents. A ligation group was prepared with ligation of the
LAD at the same site as LTD₄ infusion. Therefore, it was possible to compare myocardial
high energy phosphate contents between LTD₄ (1.0 - 3.0 μg/kg) and ligation
groups with similar levels of MBF. We found that ATP contents in the myocardium
were significantly lower in the LTD₄ groups than in the ligation group. This indicates the
presence of some MBF-independent meta-
bolic changes in the myocardium of the
LTD₄ groups.

It is well recognized that myocardial CP
levels rapidly diminish and tissue ATP con-
tents are initially preserved with acute
myocardial ischemia. Hearse et al. found
that with 5 min of ischemia, the ATP content
decreased by no more than 25%, even when the
coronary flow was reduced to almost zero²⁵ which agrees with our present results.
In clear contrast, tissue ATP levels were
significantly depressed 5 min after LTD₄ treat-
ment. Therefore, we conclude that LTD₄
could have had some effect on myocardium
other than reducing blood flow. Since no
significant difference was found between the
LTD₄ and ligation groups in terms of ATP
plus CP, the conversion of ADP + CP into
ATP + creatine might have been inhibited by
some direct action at the level of the myocardium, with consequent effects on myocardial
ATP and CP contents. Cain et al. observed
breakdown of ATP, even though CP re-
mained unchanged, when isolated frog rectus
abdominus muscles were pretreated with 1-
fluoro-2, 4-dinitrobenzene, a creatine kinase
inhibitor²³ Myocardial ATP depletion is pre-
vented during brief periods of ischemia by
utilization of CP via the creatine kinase reac-
tion³⁴,³⁵ Myocardial creatine kinase might
be inhibited by LTD₄ and this inhibition did
not result in dose-related changes in high
energy phosphate contents under the present
experimental conditions. Maximum vaso-
constriction after intracoronary injection of
LTD₄ occurs within 20 sec from the com-
encement of the injection²³,²⁴ Therefore, it
would appear that the conversion of CP to
maintain ATP levels might have been insuffi-
cient even though creatine kinase activity
was recovering 5 min after administration of
LTD₄.

While this point remains controversial,
several animal studies have indicated that
LTD₄ reduces cardiac contractility either by
direct action on cardiac muscle or through
some ischemia-dependent mechanism²³,²⁴,²⁵
On the other hand, Roth et al.³⁶ and Hahn et
al.³⁷ postulated that LTD₄ had no direct
negative inotropic activity. Such differences
of opinion may be attributed to the uncer-
tainty surrounding biochemical actions. It
remains to be determined whether the reduc-
tion of myocardial contractility which occurs
after LTD₄ administration results from a
direct negative inotropic activity of LTD₄ or
occurs secondarily via LTD₄-induced coro-
nary constriction. We did not evaluate car-
diac function, but LTD₄ might aggravate
myocardial energy metabolism with no cor-
relation to reduced myocardial perfusion.

In conclusion, infusion of LTD₄ into the
coronary artery induced a dose-related de-
crease in MBF and also brought about
changes in myocardial energy metabolism.
The fact that these two effects might be inde-
pendent suggests that LTD₄ may have some
effect on cardiac muscle other than reducing
blood flow.

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