Renal Effects of Leukotrienes C4 and D4 in Anesthetized Dogs

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Abstract—We investigated the effects of leukotrienes (LTs) C4 and D4 on renal function and hemodynamics of dogs. LTC4 (0.5 μg/min, infused into the renal artery), but not LTD4 (1 μg/min), caused an increase in renal blood flow, urine flow, urinary excretion of sodium and potassium, and rate of free water reabsorption, with a concomitant rise in systemic blood pressure. Intrarenal infusion of LTC4 under conditions of constant renal perfusion pressure induced no significant effect on renal blood flow or urine formation. Infusions of LTC4 and LTD4 into the renal artery induced no change in the release of prostaglandins, PGE2, PGF2α, 6-keto-PGF1α or thromboxane B2 into renal venous blood. From these findings, we concluded that the renal effects of LTC4 were secondary responses to a rise in systemic blood pressure.

Leukotrienes (LTs) C4 and D4, a recently discovered family of substances formed from arachidonic acid by 5-lipoxygenase, are the major constituents of the slow-reacting substance of anaphylaxis. LTs have been shown to have potent vasoactive properties. LTC4 and LTD4 causes potent constriction of vascular smooth muscles of the coronary, femoral, carotid or mesenteric arteries, in various species (1-3).

Synthesis of LTs has been reported to occur in the porcine renal artery (4) and rat glomeruli (5), and a possible role of LTs in the regulation of glomerular filtration (6, 7) has been proposed. Although several reports regarding the effects of LTs on renal hemodynamics have appeared, the renal actions of LTs remain controversial. LTC4 and LTD4 have been reported to induce a decrease (8, 9), no change (3), or an increase in renal blood flow (10). In the present study, we examined the effects of LTC4 and LTD4 on renal hemodynamics, urine formation and secretion of renin in anesthetized dogs. The effects of these LTs on the release of prostaglandins (PGs) and thromboxane (TX) were also examined to evaluate the involvement of cyclooxygenase products in the actions of LTs.

Materials and Methods

1. Experiments in the kidney: Adult mongrel dogs weighing 12 to 19 kg were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), followed by a maintenance dose (5 mg/kg, i.v.) as required. After tracheal intubation, the dogs were mechanically ventilated with room air. Catheters were inserted into the right brachial vein and the femoral artery for infusion of saline and arterial blood sampling. Systemic blood pressure was continuously monitored with a pressure transducer (Statham, P23ID, U.S.A.) via the catheter inserted into the right brachial artery. The left kidney was exposed through a retroperitoneal flank incision, and all visible nerves around the left renal artery and hilus were dissected. Renal blood flow (RBF) was measured with an electromagnetic flowmeter (Nihon Kohden, MFV-1200, Japan) equipped with automatic adjustment of zero flow base. Proximal to the flow probe, a 23-gauge hooked needle attached to a polyethylene tube was introduced into the left renal artery for intrarenal infusion of saline and test solution at a rate of 0.5 ml/min. A catheter was introduced into the left renal vein through the spermatic or ovarian vein to obtain renal
venous blood samples. The left ureter was catheterized for collection of urine samples. For measurement of the glomerular filtration rate (GFR), creatinine in a priming dose of 100 mg/kg was injected into the right brachial vein, followed by a continuous infusion at 50 mg/kg/hr. In six dogs, an adjustable clamp was placed on the aorta proximal to the left renal artery to allow for manual control of renal arterial perfusion pressure, which was monitored via the catheter inserted into the femoral artery. After completion of the surgical procedure, a period of 60–90 min was allowed for stabilization of RBF and urine flow. The experiments were carried out according to the following protocol.

Urine samples were collected during two consecutive 10 min control clearance periods. At the midpoint of each period, blood samples were collected via the catheters. After the control periods, the intrarenal infusion of saline was replaced with the solution of LTC$_4$ or LTD$_4$ and maintained for 16 min. We infused LTC$_4$ and LTD$_4$ at rates of 0.5 $\mu$g/min and 1.0 $\mu$g/min, respectively, because in the preliminary experiments, half the doses of these LTs reduced the femoral blood flow (approximately one half of RBF under basal conditions) when administered into the femoral artery. Blood samples were collected at min 5 and 15 of the infusion of LTs. Urine samples were collected from min 1 to min 8 and from min 8 to min 16 of the infusion.

2. Experiments in hindlimb: Adult mongrel dogs weighing 23 to 25 kg were used under pentobarbital anesthesia with mechanical ventilation. Catheters were inserted into the right brachial vein and artery for the infusion of saline and for the monitoring of systemic blood pressure, respectively. A femoral artery was exposed and femoral blood flow was measured with an electromagnetic flowmeter. Infusions of saline and test solution were given through a 23-gauge hooked needle introduced into the femoral artery. A catheter was inserted into the ipsilateral femoral vein through a small branch of the vein for the sampling of femoral venous blood. Arterial blood samples were collected via a catheter placed in the contralateral femoral artery. We infused LTC$_4$ and LTD$_4$ into the femoral artery at rates of 0.25 $\mu$g/min and 0.5 $\mu$g/min, respectively. Since LTD$_4$ was less active than LTC$_4$ in the preliminary experiments, a larger dose of LTD$_4$ was used. Experiments were performed using a protocol similar to that in the experiments in the kidney, except for the urine sampling.

3. Analytical procedures: For each dog, ten milliliters of blood samples were collected in precooled plastic tubes containing 20 mg of EDTA-Na$_2$ and 200 $\mu$g of meclofenamate. Collected samples were kept in melting ice and centrifuged. Measurements of PGs, TX and plasma renin activity (PRA) were made from the obtained plasma.

PRA was determined by radioimmunoassay of angiotensin I (11) and expressed as nanograms of angiotensin I per milliliter per hour. Renin secretion rate (RSR) was calculated from the renal venoarterial difference of PRA and renal plasma flow. Urinary concentrations of sodium and potassium were analyzed with a flame photometer (Hitachi, 4750, Japan). Plasma and urine osmolarity was measured with a cryoscopic osmometer (Vogel, OM-801, Giessen).

The concentrations of PGs and TX in arterial and renal venous plasma were measured by radioimmunoassay as previously reported (12, 13). Briefly, 4 ml of plasma was extracted with 30 ml of petroleum ether to remove neutral lipids. After removal of the organic phase, a 3 ml portion of the aqueous phase was extracted with a solvent mixture (9 ml) consisting of ethyl acetate-isopropyl alcohol-0.1 N HCl, 3:3:1 by volume. In the course of extraction, PGL$_2$ and TXA$_2$ were converted to 6-keto-PGF$_{1\alpha}$ and TXB$_2$, respectively. The obtained organic phase was evaporated under a stream of nitrogen at 55°C. The remaining residue was subjected to adsorption chromatography, using a silicic acid column (0.6 g, 100 mesh, Mallinkrodt, MO). The column was successively eluted with three solvent systems of benzene, ethyl acetate and methanol (solvent A, 60:40:0; solvent B, 60:40:2; solvent C, 60:40:20; by volume). The eluate was separated into three fractions. PGE$_2$ and TXB$_2$ were eluted with solvent B (second fraction), and PGF$_{2\alpha}$ and 6-keto-PGF$_{1\alpha}$ were eluted with solvent C (third fraction). Each fraction was evaporated and reconstituted with ethyl
alcohol (0.1 ml) and saline-phosphate buffer (0.9 ml, pH 7.4). Sample (0.1 ml), tritium-labeled prostanoid (0.1 ml), and appropriately diluted antiserum (0.1 ml) were incubated for 6–10 hr at 4°C. Free labeled prostanoid was separated from antibody-bound material with dextran-coated charcoal. The antiserum to 6-keto-PGF₁α and TXB₂ was kindly provided by Dr. K. Nishikawa of Takeda Chemical Industries and Dr. T. Inagawa of the Ono Pharmaceutical Company, respectively. The antiserum to 6-keto-PGF₁α cross-reacted with PGF₁α (5.9%), PGF₂α (0.9%), 6,15-diketo-PGF₁α (0.4%) and 6,15-diketo-13,14-dihydro-PGF₁α (0.3%). The antiserum to TXB₂ did not cross-react significantly with other PGs (less than 0.1%). The PGE₂ antiserum and the PGF₂α antiserum were purchased from the Pasteur Institute (Paris, France). The antiserum to PGE₂ cross-reacted with PGE₁ (6.5%) and 15-keto-PGE₂ (13.2%), and hardly did with 6-keto-PGF₁α and TXB₂ (less than 0.01%). The antiserum to PGF₂α cross-reacted with PGF₁α (12.0%), 6-keto-PGF₁α (0.04%), and hardly did with TXB₂ (less than 0.01%).

4. Materials: LTC₄ and LTD₄ (Ono Pharmaceutical Co. Ltd., Japan) were obtained as a stock solution (100 μg/ml) in 50% aqueous methanol. The stock solutions were divided into 100 μl aliquots and stored under argon at −80°C. On each experimental day, an aliquot was thawed and diluted with saline.

5. Statistical analysis: The values presented are means±S.E. A paired t-test was used only when two related sets of data were compared. For multiple comparisons, two-way analysis of variance was used. P values less than 0.05 were accepted as being statistically significant differences.

Results

1. Effects of LTs in renal and femoral hemodynamics: Effects of LTC₄ and LTD₄ on the renal hemodynamics are shown in Table 1. Intrarenal administration of LTC₄ (0.5 μg/min) resulted in a gradual increase in RBF. RBF at min 15 of the infusion was significantly increased by an average of 11%, and systemic blood pressure was significantly elevated. The renal vascular resistance was not changed during the infusion (Fig. 1). Intrarenally administered LTD₄ (1.0 μg/min) caused no change in RBF or systemic blood pressure. On the other hand, intra-femoral arterial administration of LTC₄ (0.25 μg/min) or LTD₄ (0.5 μg/min) markedly reduced the femoral blood flow without affecting systemic blood pressure (Table 2), so that the vascular resistance of hindlimb was significantly increased (Fig. 1). The vasoconstrictive effects were sustained over 15 min after the cessation of the infusion.

2. Effects of LTs on renal function and renin secretion: Figure 2 shows the effects of LTC₄ on renal function. Intrarenal administration of LTC₄ (0.5 μg/min) increased urine flow about two-fold in the second period of the infusion, with concomitant increase in the urinary excretion of sodium. Urinary excretion of potassium and the rate of free water reabsorption also significantly increased, but GFR was unaltered. Infusion of LTD₄ (1 μg/min) did not affect these parameters (data not

| Table 1. Effects of LTC₄ and LTD₄ on renal hemodynamics |
|-------------------------------------------------------|
| Systemic blood pressure (mmHg)                                      | Renal blood flow (ml/min) |
|-----------------------------|-----------------------------|
| Control (n=6)               | 125±3                        | 143±20                        |
| LTC₄ (0.5 μg/min)            |                             |                               |
| 5 min                       | 130±3*                       | 153±22*                       |
| 15 min                      | 132±3*                       | 158±21*                       |
| Control (n=6)               | 122±5                        | 139±4                         |
| LTD₄ (1.0 μg/min)            |                             |                               |
| 5 min                       | 123±5                        | 135±6                         |
| 15 min                      | 124±4                        | 135±6                         |

Asterisks denote significant differences (P<0.05) when compared with the control.
Intrarenal administration of LTC4 and LTD4 did not change renal venous PRA (control, 3.1±0.8 ng/ml; infusion period of LTC4, 2.6±0.8 ng/ml; infusion period of LTD4, 2.5±1.1 ng/ml), arterial PRA nor RSR (data not shown). Thus, these LTs did not affect renin secretion.

3. Effects of LTs on the release of PGs and TX from the kidney and the hindlimb: Table 3 shows the concentrations of PGE2, PGF2α, 6-keto-PGF1α and TXB2 in the renal and femoral venous plasma, both in the control period and at min 15 of the infusion of LTC4. Intrarenal administration of LTC4 induced no change in the concentrations of these PGs or TX in the renal venous plasma. Concentrations of PGE2, PGF2α, 6-keto-PGF1α and TXB2 in the arterial plasma in the control period were 95±35, 232±64, 270±73 and 206±101 pg/ml.
ml; and they were unchanged over the infusion period (data not shown). Although LTC₄ induced vasoconstriction of the hind-limb as described above, no change in the concentrations of PGs or TX in the femoral venous and arterial plasma was induced.

![Chart](image)

**Fig. 2.** Changes in urine flow (UF), GFR, urinary excretion of sodium (U₉₄V) and the rate of free water reabsorption (T₉Hₒ), before and at min 15 of the infusion of LTC₄. LTC₄ was infused into the renal artery at 0.5 μg/min under basal conditions (open columns, n=6) and under the condition of constant renal perfusion pressure (shaded columns, n=6). Asterisks denote significant differences (P<0.05) when compared with the control.

| Table 3. Plasma levels of PGs and TX under basal conditions and during LTC₄ infusion |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Renal vein      |                 | Femoral vein    |                 |
|                                | Control         | LTC₄            | Control         | LTC₄            |
| PGE₂ (pg/ml)                   | 102±31          | 128±39          | 70±42           | 80±47           |
| PGF₂α (pg/ml)                  | 270±64          | 288±58          | 240±68          | 214±102         |
| 6-keto-PGF₁β (pg/ml)           | 294±79          | 308±79          | 309±82          | 380±90          |
| TXB₂ (pg/ml)                   | 314±85          | 281±50          | 331±52          | 357±69          |

LTC₄ was infused into the renal (n=6) and femoral artery (n=5).
Administration of LTD₄ into the renal or femoral artery also induced no change in the venous or arterial concentrations of PGs or TX (data not shown).

4. Effects of LTC₄ under constant renal perfusion pressure: Since LTC₄ infusion into the renal artery resulted in a significant rise in systemic arterial blood pressure, renal actions of LTC₄ were examined under conditions of constant renal perfusion pressure. The renal perfusion pressure was 121±4 mmHg in the control period, and it was maintained at 119±4 mmHg using an aortic clamp, throughout LTC₄ infusion (0.5 µg/min). In this case, RBF was unchanged (control, 165±16 ml/min; infusion period, 169±20 ml/min). Urine flow, GFR, urinary excretion of sodium and rate of free water reabsorption all remained unchanged during LTC₄ infusion (Fig. 2).

Discussion

In the present experiments, intrarenal administration of LTC₄ induced a significant increase in RBF, with concomitant rise in the systemic blood pressure. LTD₄ at the dose we employed did not affect RBF, nor the systemic blood pressure. Renal vascular resistance was not changed by the intrarenal administration of LTs. Chapnick (3) reported that an injection of LTC₄, LTD₄ or LTE₄ as a bolus into the canine superior mesenteric artery produced a dose-dependent decrease in mesenteric blood flow, but these LTs induced little or no change in RBF when injected into the renal artery. Feigen (10) reported that RBF in dogs increased after injections of 3 and 10 µg of LTC₄ and LTD₄ into the renal artery. In the present study, we examined the effects of continuous infusion of LTs on renal circulation and found that LTC₄ and LTD₄ have little effect on canine renal vasculature. LTC₄ and/or LTD₄ were shown to produce vasoconstriction in the porcine and rat kidneys (9, 14, 15). However, these LTs did not affect an isolated strip of rabbit renal artery (16) and relaxed the canine renal artery (17). There seems to be species differences in the responses of the renal vascular bed to LTs.

The present study showed that LTC₄ produced profound vasoconstriction in the canine hindlimb, and that LTC₄ was more active than LTD₄. Greenwald et al. (2) reported that LTC₄ and LTD₄ induced a marked reduction in blood flow in the porcine hindlimb, and that these LTs were equipotent. On the other hand, LTD₄ has been reported to be more active than LTC₄ in producing vasoconstriction in canine mesenteric vasculature (3). Though the cause of the discrepancy in the relative potencies of LTs remains unclear, the vasoactive effects of LTs are different among organs as well as among species.

Many reports have appeared in the literature regarding the involvement of PGs and TX in the actions of LTs. It was reported that pretreatment with cyclooxygenase inhibitors modified the vasoactive effects of LTs in guinea pigs (18, 19), and LTs stimulated the release of PG₁₂ and/or TXA₂ (20, 21). Thus, in guinea pigs, the actions of LTs seem likely to depend on cyclooxygenase products (22). Piper et al. (4) reported that the vasoconstrictive effect of LTC₄ or LTD₄ in the porcine kidney was enhanced in the presence of indomethacin, suggesting the release of PG₁₂ by these LTs. On the contrary, in an isolated perfused rat kidney, the renal vasoconstriction induced by LTC₄ or LTD₄ was not inhibited by either indomethacin or OKY-1581 (a blocker of thromboxane synthesis) (8). To obtain direct evidence, we determined the concentrations of PGs and TX in the renal venous and arterial blood and found that those of PGs and TX did not change during the infusion of LTs. These findings confirm the failure of LTs to stimulate the release of PGs and/or TX from the kidney. During the infusion of LTC₄ or LTD₄ into the canine hindlimb, no change in the concentrations of PGs and TX in the femoral venous and arterial blood was detected. Consequently, we considered that LTs-induced vasoconstriction in hindlimb was not mediated by the enhancement of TX formation or suppression of PGs formation. Based on these findings, we conclude that cyclooxygenase products were not involved in these actions of LTs in dogs.

The effects of LTs on renal functions in rats have been reported. Badr et al. (14) reported that systemic infusion of LTC₄ into anesthetized rats produced a reduction in RBF and GFR. On the other hand, Filep et al. (7) reported that LTC₄ intravenously injected...
into conscious rats increased RBF, GFR, urine flow and urinary sodium excretion. In the present study, we found that intrarenal administration of LTC4 but not LTD4 resulted in an increase in urine flow, urinary excretion of sodium and potassium, and free water reabsorption without producing a change in GFR. We postulated the following possible mechanisms by which LTC4 induced these effects. First, changes in renal function induced by LTC4 would be mediated by changes in the syntheses of PGs and TX in the kidney. However, LTs produced no change of renal prostanoids syntheses, as above mentioned. Second, changes in the renin-angiotensin system might be induced by LTC4 infusion. In the present study, we investigated renin secretion during LTs infusion, but renal venous and arterial PRA and RSR were all unchanged by LTs. Further possibilities are that changes in renal function might be a consequence of a direct action of LTC4 on the renal tubulus, or a secondary response induced by a systemic pressor effect of LTC4. Evidence supporting the latter assumption was obtained by the experiment which examined the effects of LTC4 under constant renal perfusion pressure. In this experiment, we were able to exclude the systemic effect of LTC4, that is, a rise in the systemic blood pressure. The increase in urine flow, urinary excretion of sodium and potassium, and free water reabsorption due to LTC4 infusion were abolished under these conditions. We conclude that the LTC4-induced changes in renal function were secondary responses to a rise in the systemic blood pressure (23) or to an increase in RBF (24).

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