PREVENTION OF ENDOGENOUS LEUKOTRIENE PRODUCTION DURING ANAPHYLAXIS IN THE GUINEA PIG BY AN INHIBITOR OF LEUKOTRIENE BIOSYNTHESIS (MK-886) BUT NOT BY DEXAMETHASONE

By ALBRECHT GUHLMANN,* ANDREA KEPPLER,* STEFANIE KÄSTNER,* HEINER KRIETER,‡ UWE B. BRÜCKNER,‡ KONRAD MESSMER,‡ AND DIETRICH KEPPLER*

*From the *Division of Tumor Biochemistry, Deutsches Krebsforschungszentrum, and the ‡Department of Experimental Surgery, University of Heidelberg, D-6900 Heidelberg, Federal Republic of Germany*

The cysteinyl leukotrienes (LTs),[1] LTC_4, LTD_4, and LTE_4, are arachidonate metabolites generated in the 5-lipoxygenase pathway (1–3). Full activation of 5-lipoxygenase requires a Ca^{2+}-dependent translocation of the enzyme from the cytosol to a membrane-bound site (4–6). Inhibition of this process was shown to prevent LT generation (6, 7). LTC_4, which is formed by conjugation of the 5-lipoxygenase product LTA_4 with glutathione, is rapidly metabolized within the vascular space by the ectoenzymes γ-glutamyltransferase and, in most species, dipeptidase (8), yielding LTD_4 and LTE_4, respectively (9–11). LTC_4, LTD_4, and LTE_4 are eliminated from the blood circulation with initial half-lives of 30–40 s (9, 10, 12–14), mainly taken up by the liver and excreted into bile (9, 10, 12–17). A portion of the cysteinyl LTs eliminated with bile undergoes enterohepatic circulation (9, 18).

A limited number of cell types, including mast cells, eosinophils, and mononuclear phagocytes, are capable of producing LTC_4 (2, 19–22), which is presumed to play a major role as a mediator of hypersensitivity reactions and inflammation (1–3, 23). In guinea pigs, intravenously administered LTC_4 or LTD_4 provoke severe respiratory distress as well as the typical hemodynamic changes observed in systemic anaphylaxis (23, 24). Enhanced production in vivo of endogenous LTC_4 was demonstrated in acute anaphylaxis (16) and under several other pathophysiological conditions (25), including tissue trauma (12), endotoxin shock (17), shock induced by staphylococcal enterotoxin B (9), hepatorenal syndrome (26), infusion of TNF-α (27), and injection of platelet-activating factor (PAF) (10).

A variety of humoral mediators, including the LTs (16, 28), histamine (29), PAF (30), and prostanoids (31, 32), are involved in the pathogenesis of systemic anaphylaxis.

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Address correspondence to Dietrich Keppler, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, D-6900 Heidelberg 1, FRG.

[1] *Abbreviations used in this paper:* C_{dyn}, dynamic lung compliance; HTMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine-N(1)-oxyl; LT, leukotriene; MAP, mean arterial pressure; PAF, platelet-activating factor; RP-HPLC, reversed-phase HPLC.

Guinea pigs suffering from anaphylactic shock produce LTD_4 endogenously in amounts that may be sufficient to induce cardiovascular and hemodynamic effects leading to severe shock reactions in this species (16). Therefore, inhibitors of LT synthesis as well as LT receptor antagonists (33) may be of therapeutic value in the treatment of anaphylactic shock. Since the antiinflammatory glucocorticosteroids are potent antiasthmatic drugs, we set out to test the possibility that dexamethasone prevents symptoms of systemic anaphylaxis in the guinea pig by inhibiting the release of LTs in vivo. We demonstrate the suppression of cysteinyl LT generation in vivo during anaphylactic shock by the novel LT biosynthesis inhibitor MK-886 (previous code number, L-663,536; chemical name, 3-[1-(4-chlorobenzyl)-3-*t*-butylthio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid) (7). In animals pretreated with the histamine H_1 receptor antagonist pyrilamine, inhibition of whole body LTC_4 generation is associated with a protection from death and other consequences of acute systemic anaphylaxis.

Materials and Methods

Special Reagents. Unlabeled LTC_4, LTD_4, and LTE_4 were purchased from Miles Scientific through Bayer Diagnostik (München, FRG). [14,15-$^3H_2$]LTC_4, [14,15-$^3H_2$]LTD_4, and [14,15-$^3H_2$]LTE_4 (40 Ci/mmol or 1.5×10^{15} Bq/mol, each) were obtained from New England Nuclear (Boston, MA). *N*-Acetyl-LTE_4 and *N*-acetyl-[^{3}H]LTE_4 were synthesized from LTE_4 and [^{3}H]LTE_4, respectively, using acetic anhydride (15). Reversed-phase HPLC (RP-HPLC) separation served to control the purity of the LTs and to purify the unlabeled LTs (9). The concentration of unlabeled LTs was determined by absorbance measurements at 280 nm using an extinction coefficient of 40,000 $cm^{-1} \times M^{-1}$ (34). 6-Keto [5,8,9,11,12,14,15-$^3H_7$] $PGF_{1\alpha}$, sp act 157 Ci/mmol or 5.81×10^{15} Bq/mol, and the 6-keto-$PGF_{1\alpha}$ assay system were from Amersham Buchler (Braunschweig, FRG). 4-Hydroxy-2,2,6,6-tetramethylpiperidine-*N*(1)-oxyl (HTMP), L-penicillamine, OVA (grade III), pyrilamine maleate, Evans' blue, methyl cellulose, zymosan A, γ-glutamyltransferase, and activated charcoal were obtained from Sigma Chemical Co. (St. Louis, MO); dexamethasone 21-dihydrogenphosphate (Fortecortin) was from E. Merck (Darmstadt, FRG); dextran T 70 (~70 kD) from Pharmacia Fine Chemicals (Uppsala, Sweden); ketamine (Ketavet) from Parke-Davis (München, FRG); and xylazine (Rompun) from Bayer Leverkusen (Leverkusen, FRG). PBS and Eagle's MEM were obtained from Boehringer Mannheim Biochemicals (Mannheim, FRG), and supplemented with L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 mg/ml) (Gibco Laboratories, Grand Island, NY). Dr. G. Neil, Upjohn Co. (Kalamazoo, MI), kindly supplied acivicin. MK-886 (L-663,536) sodium salt, was a generous gift from Drs. J. Rokach and J. Gillard, Merck Frosst Canada (Pointe-Claire/Dorval, Quebec, Canada). The monoclonal cysteinyl LT antibody was kindly donated by Dr. F. Kohen, The Weizmann Institute of Science (Rehovot, Israel).

Animal Experiments. Male guinea pigs of the Hartley strain (Interfauna Süddeutsche Versuchstierfarm, Tuttlingen, FRG) weighing 400–600 g were actively sensitized to OVA 3 wk before antigen challenge by intraperitoneal injection of 3 μg/kg OVA mixed with 300 mg/kg $Al(OH)_3$ to produce both IgE- and IgG1-like antibodies (35). Cutaneous testing of the sensitization was performed in each animal by examination of the local extravasation of intravenously injected Evans blue dye (10 mg/kg, dissolved in saline) after intradermal injection of OVA (1 μg). Sensitized guinea pigs were defined as those showing a blue area of at least 6 mm in diameter at the site of OVA injection. Animals were anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). After a median laparotomy with an incision length of 2 cm, the cystic duct was ligated, the gall bladder punctured, and the bile duct cannulated (polyethylene tubing, 1.0-mm outer diameter, 0.6-mm inner diameter; Dow Corning Corp., Midland, MI) (16). The animal's core temperature was maintained at 37 ± 1°C with a heating pad. After cannulation of both jugular veins and of the left carotid artery,

the animals were kept under anesthesia for 3 h to avoid an interference of the LT measurements by LTs generated during the trauma required for the surgical interventions (12). Anaphylaxis was induced by intravenous administration of 0.2 mg/kg OVA dissolved in saline. For metabolic experiments, [$^3H_2$]LTC_4 (5 μCi/kg) was either injected intravenously as a bolus or infused into the jugular vein over a period of 15 min in sensitized animals exposed to antigen challenge as well as in sensitized controls. Arterial blood was withdrawn into a syringe containing PBS with 3 mM EDTA, 2 mM acivicin, 2 mM L-penicillamine, 1 mM HTMP (final concentrations) at pH 7.4 (10). Blood samples were mixed immediately with 4 vol of ice-cold methanol containing 1 mM HTMP. Bile was collected continuously under argon into ice-cold 90% (vol/vol) aqueous methanol, containing 1 mM HTMP and 0.5 mM EDTA at pH 7.4.

Respiratory and Hemodynamic Parameters. A small polyethylene catheter (0.3-mm inner diameter; Dow Corning) was passed via the left carotid artery into the aorta for pressure measurements, monitoring of the heart rate (BioTach, Gould-Brush, Cleveland, OH), and blood sampling. Two additional polyethylene catheters (0.6-mm inner diameter) were introduced into both external jugular veins for drug and anesthetic administration. Arterial blood gases were analyzed by ABL 3 equipment (Radiometer, Copenhagen, Denmark). The animals were tracheotomized and a metal tube (2.6-mm inner diameter) was inserted for artificial ventilation (Animal Respirator Type 4600; Rhema Labortechnik, Hofheim, FRG) with an air/O_2 mixture (F_IO_2, 30%; arterial pCO_2, 4.7–5.3 kPa). No positive endexpiratory pressure was used. Respiratory pressures were measured through the tube and by a 26-gauge needle inserted into the trachea 5 mm below the tube. All pressures were monitored via Statham P23ID pressure transducers calibrated manometrically before each experiment; the zero level was set at midchest position. The cardiovascular and respiratory variables were simultaneously recorded on a multi-channel polygraph (Mk 481; Brush, Cleveland, OH). The dynamic lung compliance (C_{dyn}) was calculated as: tidal volume/(respiratory peak pressure – endexpiratory pressure).

Drug Administration. The animals received the histamine antagonist pyrilamine maleate, dissolved in saline, at 1 mg/kg, i.v., 5 min before the immunologic challenge with OVA. Dexamethasone was administered either over a period of 7 d (10 mg/kg daily, i.p., the last dose was given intravenously 1 h before the challenge with OVA) or at a single dose of 10 mg/kg i.v., 3.5 h before immunological challenge. MK-886 was given at a dose of 1 or 10 mg/kg, i.v., 15 min before OVA. MK-886 was first dissolved in absolute ethanol (200 mg/ml) and then diluted with a 0.1% aqueous solution of methyl cellulose to 10 mg/ml. OVA challenge controls received the vehicle alone. Unlabeled LTC_4 was infused intravenously over a period of 5 min in a total dose of 2 nmol/kg.

Guinea Pig Peritoneal Macrophage Culture. Before the surgical manipulations described above, resident peritoneal macrophages were isolated from dexamethasone-treated (10 mg/kg, i.p., once daily for 7 d) and from untreated sensitized guinea pigs by lavage with sterile PBS. 1–2 × 10^6 peritoneal macrophages were plated on 5-cm-diameter plastic tissue culture dishes (Becton Dickinson & Co., Mountain View, CA) in 3 ml of Eagle's MEM containing 5% FCS for 2 h at 37°C under 5% CO_2/95% air. After removal of nonadherent cells by washing the dishes twice with PBS, the adherent cells were placed in 2 ml of serum-free Eagle's MEM containing 50 μg/ml of unopsonized zymosan prepared by the method of Bonney et al. (36). The cells were then cultured for 2 h at 37°C under 5% CO_2/95% air. The cell supernatants were removed and passed through Sep-Pak cartridges containing octadecylsilyl silica (10 × 10 mm; Waters Associates, Milford, MA) that had been prewashed sequentially with 50 ml each of methanol, water, methanol/water (1:9, vol/vol) containing 14 mM EDTA, pH 7.4, and water. After applying the samples, the columns were rinsed successively with 5 ml of water, 15% (vol/vol) aqueous methanol, and benzene; LTs and PGs were then eluted with 2 ml of absolute methanol. Using this procedure, recoveries of ^{3}H-labeled cysteinyl LTs and of ^{3}H-labeled 6-keto-$PGF_{1\alpha}$ were at 85 ± 5% and 82 ± 3%, respectively. Aliquots of the methanol fractions were dried under vacuum and arachidonate metabolites were analyzed by RIA.

RP-HPLC. For deproteinization before application to HPLC, biological samples were made of 80% methanol containing 1 mM HTMP, stored at −30°C for at least 3 h, and

centrifuged at 10,000 *g* for 10 min at 4°C. For analyses of endogenous cysteinyl LTs, aliquots of the supernatants equivalent to 25 μl of bile were mixed with 800 cpm each of [^{3}H]LTC_4, [^{3}H]LTD_4, [^{3}H]LTE_4, and *N*-acetyl-[^{3}H]LTE_4 to correct for recovery losses in the analysis of endogenous LTs. The samples were dried in a SpeedVac concentrator (Savant Instruments, Inc., Hicksville, NY) and resuspended in 30% (vol/vol) aqueous methanol. RP-HPLC was performed isocratically on a C18-Hypersil column (4.6 × 250 mm, 5-μm particles; Shandon, Runcorn, UK) with a C18 precolumn (Waters Associates). The mobile phase consisted of 65% (vol/vol) methanol in aqueous buffer (0.1% acetic acid, 1 mM EDTA, pH 5.6, adjusted with ammonium hydroxide). The flow rate was 1 ml/min. For analyses of the [^{3}H]LT metabolite pattern in blood and bile, tritium in the HPLC eluent was detected continuously with a liquid scintillation device (LB 505; Berthold, Wildbad, FRG) using a Rialuma scintillation mixture from Baker Chemicals (Gross-Gerau, FRG). For analyses of endogenous cysteinyl LTs, a 400-μl aliquot of each 1-ml fraction collected during HPLC was counted for calculation of tritium recovery, and 600 μl was dried for the subsequent RIA. The amounts of immunoreactive LT metabolites coeluting with the [^{3}H]LT standards added to each sample before HPLC were corrected for recovery losses and immunologic crossreactivity in the RIA.

RIA for Cysteinyl LTs. The assay procedure is a modification of the one described previously (9). A 600-μl aliquot of the HPLC fractions, as well as evaporated aliquots of macrophage supernatants prepared as described above and resuspended in 600 μl of HPLC eluate, were evaporated to dryness and resuspended in 200 μl of assay buffer (0.9% NaCl, 0.1% gelatin, 10 mM EDTA, 0.1% sodium azide in 10 mM phosphate buffer, pH 7.4). Tubes for LTC_4, LTD_4, and LTE_4 standard curves also contained 600 μl of dried, LT-free HPLC eluate. Antibody, diluted $1:10^5$ in assay buffer, was added to the samples and standards. After mixing and a 30-min preincubation period at room temperature, [^{3}H]LTE_4 (6,000 cpm) was added in 100 μl of assay buffer, mixed, and incubated at 4°C for 16–20 h. Unbound [^{3}H]LTE_4 was removed by addition of 1 ml of ice-cold charcoal suspension (0.63% charcoal, 0.063% dextran in 10 mM phosphate buffer, pH 7.4) and, after 4 min, by centrifugation at 1,400 *g* for 15 min at 4°C. The supernatant was added to 10 ml of scintillation fluid for counting of radioactivity. The lower detection limit of the assay system for LTD_4 was at ~13 fmol. The molar crossreactivities at 50% binding of LTE_4, *N*-acetyl-LTE_4, LTD_4, and LTC_4 were 100, 140, 160, and 210%, respectively.

RIA for 6-keto-prostaglandin $F_{1\alpha}$. The assay system provided by Amersham Buchler was used. The 6-keto-$PGF_{1\alpha}$ antibody crossreacted with prostanoids as follows: 6-keto-$PGF_{1\alpha}$, 100%; PGE_2, 5.1%; $PGF_{2\alpha}$, 0.30%; PGD_2, <0.014%; thromboxane B_2, <0.014% (relative molar crossreactivities at 50% binding). The lower detection limit for 6-keto-$PGF_{1\alpha}$ was at ~40 fmol.

Enzymatic Modification of Endogenously Formed LTC_4. Deproteinized bile collected 0–15 min after OVA challenge was mixed with 800 cpm of [^{3}H]LTC_4 as standard, evaporated to dryness, resuspended in 30% (vol/vol) aqueous methanol, and fractionated by RP-HPLC. Fractions coeluting with [^{3}H]LTC_4 were evaporated to dryness, dissolved in PBS (pH 7.0) containing γ-glutamyltransferase (50 mU/ml) and L-penicillamine (2 mM) (10), and incubated for 30 min at 37°C. After deproteinization in 80% (vol/vol) aqueous methanol and centrifugation, the supernatants were evaporated to dryness, redissolved in 30% (vol/vol) methanol, and subjected to RP-HPLC. Fractions were analyzed for LTD_4 by RIA as described above.

Statistical Analyses. Data from different experiments were combined and reported as the mean ± SEM. Student's two-tailed *t* test was used to analyze differences for significance. Normal distribution was ascertained and the Bonferroni correction was applied. Significant differences in the lethality studies (Table II) were calculated using Woolf's *G* test.

Results

Elimination and Metabolism of Labeled LTC_4 after its Intravenous Injection or Infusion. [^{3}H]LTC_4, injected intravenously as a bolus, underwent rapid elimination from the circulating blood with an initial half-life of the radioactivity of 38 ± 4 s. Extensive conversion of [^{3}H]LTC_4 to [^{3}H]LTD_4 occurred within the vascular space

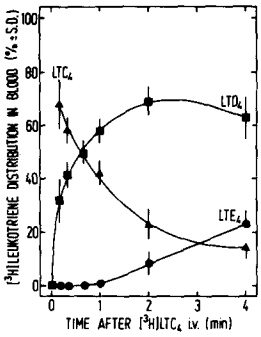

FIGURE 1. Distribution of cysteinyl LTs in the blood circulation after intravenous injection of [^{3}H]LTC$_4$ (5 μCi/kg) as a bolus. Blood samples were collected and immediately mixed with a cold solution containing EDTA, acivicin, and L-penicillamine as described in Materials and Methods. Acivicin and L-penicillamine served to inhibit γ-glutamyltransferase and LTD$_4$ dipeptidase, respectively, and thereby prevent further catabolism of LTC$_4$ and LTD$_4$ (10). [^{3}H]LTs in blood were separated by RP-HPLC and expressed as relative percentages. Mean values from three guinea pigs ± SD are given.

(Fig. 1). 68 ± 3% of the radioactivity in arterial blood coeluted with standard [^{3}H]LTD$_4$ as analyzed by RP-HPLC of samples collected 2 min after intravenous injection of [^{3}H]LTC$_4$. Only a slow conversion to [^{3}H]LTE$_4$ was observed in the circulation. The initial half-life of [^{3}H]LTC$_4$ itself was 15 s in the vascular bed of the guinea pig and resulted from both hepatobiliary elimination and conversion to [^{3}H]LTD$_4$.

The hepatobiliary elimination of cysteinyl LTs and the biliary metabolite pattern were analyzed after intravenous infusion of [^{3}H]LTC$_4$ over a 15-min period. Within 30 min, 67 ± 6% of the infused radioactivity was recovered in bile (Fig. 2). HPLC analyses indicated that [^{3}H]LTD$_4$ and [^{3}H]LTC$_4$ represented the major metabolites in bile amounting to 46 ± 1% and 18 ± 4%, respectively, of the infused dose within 30 min. Only small amounts of tracer corresponded to biliary [^{3}H]LTE$_4$ and [^{3}H]LT metabolites more polar than [^{3}H]LTC$_4$. Intravenous infusion of [^{3}H]LTC$_4$, together with unlabeled LTC$_4$ (2 nmol/kg), showed a similar biliary metabolite profile. In these animals LTC$_4$ caused a 37% (n = 4; $p < 0.005$) decrease in bile flow during a 15-min period after infusion. No significant differences in either the biliary excretion rate or in the biliary LT metabolite pattern were observed between sensitized animals exposed to antigen challenge and sensitized controls.

Influence of LT Biosynthesis Inhibition on Systemic LTC$_4$ Generation In Vivo during

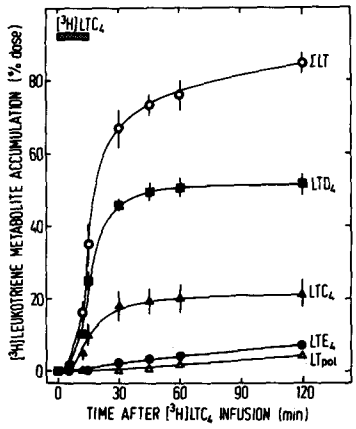

FIGURE 2. [^{3}H]LT metabolites accumulated in bile during and after intravenous infusion over 15 min of [14,15-^{3}H$_2$]LTC$_4$. Biliary [^{3}H]LT metabolites were separated by RP-HPLC and defined by their retention times and coelution with [^{3}H]LT standards. The amount of the metabolites accumulated in bile is expressed as percent of the infused dose. LT$_{pol}$ designates [^{3}H]LT metabolites more polar than [^{3}H]LTC$_4$; Σ LT represents total radioactive LT metabolites excreted into bile. Mean values from four animals ± SEM are given.

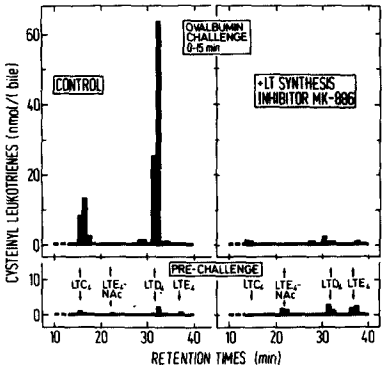

FIGURE 3. Effect of the LT biosynthesis inhibitor MK-886 on antigen-induced cysteinyl LT generation in vivo. Biliary cysteinyl LTs were separated by RP-HPLC and fractions were analyzed by RIA as described in Materials and Methods. Arrows indicate retention times of internal [^{3}H]LT standards added to each sample before HPLC in small amounts not interfering with the RIA. (*Lower panels*) Radioimmunochromatograms of bile samples collected 30–0 min before antigen challenge. (*Upper panel, left*) Analysis of bile sample collected 0–15 min after OVA challenge. (*Upper panel, right*) Suppression of antigen-induced rise in cysteinyl LT concentration in the postchallenge sample of guinea pig pretreated with the LT biosynthesis inhibitor MK-886 (10 mg/kg, i.v.) 15 min before challenge.

Anaphylaxis. Biliary cysteinyl LTs were analyzed during anaphylactic shock, induced by intravenous injection of OVA (0.2 mg/kg) into sensitized guinea pigs, by the combined use of RP-HPLC and subsequent RIA. A radioimmunochromatogram of bile samples collected before and after OVA challenge is shown in Fig. 3. In accordance with previous studies (16), LTD_4 was the major endogenous immunoreactive LTC_4 metabolite in anaphylactic guinea pig bile. Antigen challenge was followed by a 30-fold increase in the biliary LTD_4 concentration from 2.1 ± 0.6 nmol/liter bile to 63 ± 7 nmol/liter (n = 10) (Fig. 4). In line with the tracer studies (Fig. 2), an additional endogenous metabolite coeluting with LTC_4 was observed (Fig. 3). Biliary LTC_4 levels increased from 1.2 ± 0.9 nmol/liter bile to 33 ± 11 nmol/liter after OVA challenge. The identity of the LTC_4 was confirmed by enzymatic conversion by γ-glutamyltransferase to LTD_4 followed by rechromatography and radioimmunologic analysis. [^{3}H]LTC_4, added as an internal standard, was also completely converted to [^{3}H]LTD_4. The bile flow decreased from 115 ± 5 $\mu l \times min^{-1} \times kg^{-1}$ in prechallenge fractions to 85 ± 4 $\mu l \times min^{-1} \times kg^{-1}$ (n = 10; $p < 0.05$) in postchallenge fractions collected 0–15 min after OVA. Based on the total recovery of

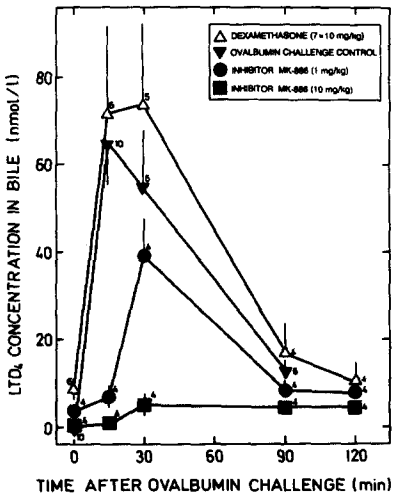

FIGURE 4. Time course of LTD_4 levels in bile from OVA-challenged, pyrilamine-pretreated guinea pigs with or without pretreatment with the LT biosynthesis inhibitor MK-886 or dexamethasone. The inhibitor was given intravenously 15 min before challenge at the indicated doses; dexamethasone was administered intraperitoneally over a period of 7 d (10 mg/kg daily). Systemic LTC_4 production was followed by measurement of the LTD_4 concentration in bile samples collected during 30 min before and during several time intervals after antigen challenge. Each LTD_4 value was determined by RP-HPLC and subsequent RIA as in Fig. 3, and is presented as the mean ± SEM. In challenge controls and in dexamethasone-treated guinea pigs, the number of animals, shown next to the symbols, decreased with time due to early death.

LTC_4 (75 ± 5%) and LTD_4 (71 ± 3%), and on the finding that within 30 min, 46 and 18% of the infused [^{3}H]LTC_4 were recovered from bile as [^{3}H]LTD_4 and [^{3}H]LTC_4, respectively (Fig. 2), the total amount of LTC_4 produced during anaphylaxis within a 30-min period was calculated to be 0.8 ± 0.2 nmol/kg (n = 10).

The LT biosynthesis inhibitor MK-886, at a dose of 10 mg/kg, blocked the rise of the LTD_4 concentration in bile (Figs. 3 and 4) as well as of the LTD_4 production rate (Table I) by >92% ($p < 0.001$) within the 30-min period after OVA challenge. MK-886 also prevented the anaphylaxis-induced decrease in bile flow observed in the OVA challenge controls. Pretreatment with a lower dose of MK-886 (1 mg/kg, i.v., 15 min before challenge) resulted in a significant reduction ($p < 0.001$) of biliary LTD_4 as compared with OVA challenge controls in samples collected 0–15 min after antigen challenge (Fig. 4). However, no significant decrease in LTD_4 levels in bile was observed at later time periods. At both inhibitor doses most of the pyrilamine-pretreated guinea pigs were protected from early death caused by the OVA-induced anaphylactic shock (Fig. 4; Table II). Moreover, symptoms usually following the OVA challenge, such as acute respiratory distress, cyanosis, and micturation, were abolished by the pretreatment with MK-886.

Influence of Dexamethasone on Eicosanoid Production. Pretreatment of guinea pigs with dexamethasone (10 mg/kg, i.v., 3.5 h before OVA challenge) did not inhibit the antigen-induced cysteinyl LT production (Table I). Moreover, long-term administration of high dexamethasone doses (10 mg/kg, i.p., once daily for 7 d) did not suppress the anaphylaxis-induced LTC_4 production in vivo as measured by the LTD_4 concentration in bile (Fig. 4). To further investigate the action of the steroid on eicosanoid release, we isolated resident peritoneal macrophages from sensitized guinea pigs pretreated with dexamethasone in vivo for 7 d (10 mg/kg daily, i.p.). Macrophages harvested from untreated sensitized guinea pigs served as controls. Exposure to zymosan was followed by a 36-fold increase in LTC_4 production and by a 13-fold increase in 6-keto-$PGF_{1\alpha}$ release (Table III). Dexamethasone pretreatment in vivo induced an 88% inhibition of LTC_4 generation as well as a 61% suppression of 6-

TABLE I

Influence of Dexamethasone and of the LT Biosynthesis Inhibitor MK-886 on the LTD_4 Production Rate during Anaphylactic Shock

Time interval	Control	Dexamethasone*	MK-886‡
min	*pmol LTD_4 × min^{-1} × kg^{-1}*		
-30–0	0.3 ± 0.1 (10)	0.6 ± 0.2 (5)	0.2 ± 0.1 (4)
0–15	6.2 ± 1.8 (10)	6.4 ± 2.9 (5)	0.3 ± 0.1 (4)§
15–30	5.2 ± 1.5 (6)	3.3 ± 1.3 (5)	0.6 ± 0.3 (4)§
30–90	1.8 ± 0.8 (6)	1.4 ± 0.5 (5)	0.5 ± 0.2 (4)

The LTD_4 production rate was calculated from the biliary LTD_4 concentration and from bile flow during the time periods before and after antigen challenge as indicated. Values are means ± SEM. The number of animals (given in parentheses) decreased with time in the challenge controls due to early death.

* Dexamethasone was given at a single dose of 10 mg/kg, i.v., 3.5 h before OVA challenge.

‡ MK-886 was administered at a dose of 10 mg/kg, i.v., 15 min before challenge.

§ Statistically significant as compared with OVA challenge controls ($p < 0.001$).

TABLE II

Effect of Inhibitors on Anaphylactic Death in Sensitized Guinea Pigs

Treatment before OVA challenge	Lethality	Number of guinea pigs
	%	
None	100	8
Pyrilamine*	78	9
MK-886 (10 mg/kg)§	60‡	10
Pyrilamine* + MK-886 (1 mg/kg)§	25‡	8
Pyrilamine* + MK-886 (10 mg/kg)§	0‡	10
Pyrilamine* + dexamethasone‖	33‡	6

Lethality caused by systemic anaphylaxis was recorded up to 2 h after intravenous injection of OVA (0.2 mg/kg) into sensitized guinea pigs. All animals were anesthetized as described in Materials and Methods.

* The histamine H_1 antagonist pyrilamine maleate was given intravenously at a dose of 1 mg/kg 5 min before OVA challenge.

‡ Statistically different from group without treatment before OVA challenge by $p < 0.05$ (Woolf's G test).

§ The LT biosynthesis inhibitor MK-886 was administered intravenously 15 min before challenge.

‖ Dexamethasone was given intraperitoneally to sensitized guinea pigs over a period of 7 d (10 mg/kg daily).

keto-$PGF_{1\alpha}$ release in zymosan-stimulated macrophages (Table III). RIAs after RP-HPLC separation indicated that ~60% of the immunoreactive cysteinyl LTs released by the macrophages corresponded to LTC_4, whereas LTD_4 and LTE_4 amounted to ~20% each.

Respiratory and Hemodynamic Parameters under the Influence of Eicosanoid Synthesis Inhibitors during Systemic Anaphylaxis. Guinea pigs, pyrilamine pretreated, artificially ventilated, and challenged with OVA, showed a 56% decrease ($p < 0.05$) in the C_{dyn}

TABLE III

Effect of Dexamethasone on Eicosanoid Production by Stimulated Peritoneal Macrophages

Treatment	Cysteinyl LTs	6-keto-$PGF_{1\alpha}$
	pmol/10^6 cells	
None	2.3 ± 0.3 (4)	8.4 ± 1.5 (4)
Zymosan*	82.5 ± 5.4 (4)	105.7 ± 9.8 (4)
Dexamethasone‡ + zymosan*	9.5 ± 3.5 (6)§	40.8 ± 7.4 (6)§

Resident peritoneal macrophages were isolated from dexamethasone-treated sensitized as well as from untreated sensitized guinea pigs. 2-h adherent peritoneal macrophage cultures were incubated with zymosan, and arachidonate metabolites were analyzed by RIA as described in Materials and Methods. Values shown for cysteinyl LTs are LTC_4 pmol equivalents. All values are means ± SEM. The number of experiments is given in parentheses.

* Zymosan was added to macrophage cultures at 50 μg/ml.

‡ Dexamethasone was given i.p. to sensitized guinea pigs over a period of 7 d (10 mg/kg daily).

§ Statistically significant as compared with zymosan-stimulated macrophages harvested from untreated sensitized guinea pigs ($p < 0.001$).

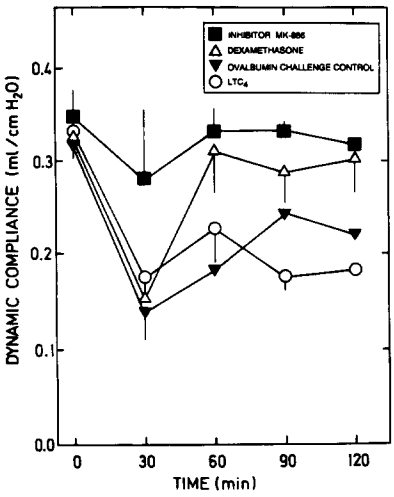

FIGURE 5. Dynamic lung compliance in OVA-challenged, pyrilamine-pretreated guinea pigs with or without pretreatment with the LT biosynthesis inhibitor MK-886 or dexamethasone, as well as in sensitized guinea pigs after LTC_4 infusion. The inhibitor was given intravenously in a dose of 10 mg/kg 15 min before the antigen was injected at t = 0; dexamethasone was administered intraperitoneally over a period of 7 d (10 mg/kg daily). C_{dyn} was measured and calculated as described in Materials and Methods. LTC_4 (2 nmol/kg) was infused over 5 min into four animals. The experimental groups challenged with OVA were composed of five animals each. Mean values ± SEM are given.

(Fig. 5). The C_{dyn} was significantly improved ($p < 0.05$) by additional pretreatment of the animals with the LT biosynthesis inhibitor MK-886 (10 mg/kg). Dexamethasone (10 mg/kg, i.p., once daily for 7 d) did not prevent the early OVA-induced decrease in C_{dyn}, but after 60 min, C_{dyn} was normalized (Fig. 5). The infusion of LTC_4 (2 nmol/kg) into sensitized guinea pigs caused a 47% decrease in C_{dyn} ($p < 0.05$), corresponding to the above-mentioned decrease in the challenged control animals.

After OVA challenge the hematocrit increased in all animals within 60 min ($p < 0.05$). This rise was most pronounced in the challenge control group (+53%). MK-886, but not dexamethasone, significantly suppressed ($p < 0.05$) the increase in hematocrit. The infusion of LTC_4 caused a 25% increase in hematocrit within 60 min ($p < 0.05$). In OVA-challenged controls, the mean arterial pressure (MAP) decreased from 56 ± 4 (prechallenge value) to 25 ± 4 mm Hg within 30 min after challenge ($p < 0.05$). No significant changes in either MAP or in the heart rate were observed in dexamethasone- or MK-886-pretreated animals 30 min after OVA challenge. Infusion of LTC_4 into sensitized guinea pigs caused a decrease in MAP from 54 ± 1 (prechallenge value) to 39 ± 3 mm Hg within 30 min ($p < 0.05$), as well as a 15% decrease in heart rate.

Discussion

This study was aimed at an inhibition of systemic cysteinyl LT production in vivo. Moreover, it was of interest whether the suppression of LT generation could reduce the life-threatening manifestations of systemic anaphylaxis. Recently, an animal model has been developed that enables measurements of systemic LTC_4 formation during anaphylactic shock in the guinea pig (16). We have used this model to evaluate in vivo the efficacy of dexamethasone and of the novel and potent inhibitor of 5-lipoxygenase activation and LT biosynthesis MK-886 (7). This inhibitor is selective for the 5-lipoxygenase pathway of arachidonate metabolism as demonstrated by the lack of effect on 12-lipoxygenase, 15-lipoxygenase, or cyclooxygenase (7).

As the basis for measurements of the systemic production of endogenous cysteinyl

LTs, [^{3}H]LTC_4 served as the precursor for determination of the metabolite pattern of LTs in blood and bile. In the guinea pig, elimination of intravenously injected [^{3}H]LTC_4 from the blood circulation was as fast as in the monkey (9, 11) and in the rat (10, 12–14). LTC_4 was rapidly converted within the vascular space to the most biologically potent cysteinyl LT, LTD_4, which was rather slowly metabolized to LTE_4 (Fig. 1). This is in contrast to LTC_4 metabolism in the monkey and the rat, where LTE_4 is the predominant metabolite in circulating blood as early as 1 min after intravenous injection of LTC_4 (9–11). The limited degradation of LTD_4 to LTE_4 within the vascular space in the guinea pig may be related to the high sensitivity of this species to cysteinyl LTs (24). Only 1 nmol/kg of intravenous LTC_4 or LTD_4 evoked widespread plasma extravasation (37). Cysteinyl LT-induced plasma extravasation around bile ducts, as indicated by means of Evans blue (37), may be responsible for the significant decrease in bile flow that we observed in sensitized guinea pigs after infusion of LTC_4, as well as during anaphylactic shock.

Antigen provocation of OVA-sensitized guinea pigs was associated with a large increase in the biliary excretion of LTD_4 (16) and, to a lesser extent, of LTC_4 under our conditions (Fig. 3). Calculation of the systemic LTC_4 production in vivo was based on the predominant hepatobiliary elimination and the biliary metabolite pattern after intravenous infusion of labeled LTC_4 over a 15-min period (Fig. 2). The infusion served to mimic the time course of endogenous LTC_4 production during anaphylaxis (Fig. 4, Table I). It should be noted that the guinea pigs, under our experimental and feeding conditions, showed a minimal capacity for ω oxidation of LTE_4, thus forming only small amounts of biologically inactive, polar LT metabolites (Fig. 2). In a different environment (16), polar LTE_4 metabolites can comprise a larger fraction.

The antigen-induced LTC_4 production was fully suppressed after pretreatment with the LT biosynthesis inhibitor MK-886 (Figs. 3 and 4, Table I). This compound does not modify the hepatobiliary elimination of cysteinyl LTs (7). Prevention of the anaphylactic LTC_4 production protected the pyrilamine-pretreated animals against lethal shock (Fig. 4; Table II), and significantly reduced the antigen-induced decrease in C_{dyn} (Fig. 5), as well as the rise in hematocrit. Moreover, infusion of LTC_4 or LTD_4 into guinea pigs caused changes in C_{dyn} (23), mean systemic arterial pressure (23), and hematocrit (37) similar to those observed during anaphylactic shock (Fig. 5). This indicates that LTs play an important role in this model of anaphylaxis.

Pretreatment with dexamethasone at various doses did not inhibit the antigen-induced cysteinyl LT production in vivo (Fig. 4, Table I). However, dexamethasone pretreatment in vivo induced a strong inhibition of cysteinyl LT generation in zymosan-stimulated resident peritoneal macrophages (Table III). These findings indicate that the glucocorticosteroid was active in the guinea pig macrophages but not in the cells responsible for the anaphylactic LTC_4 release in vivo. In the model of anaphylaxis used in the present investigation (35), mast cells are likely to be the main source of endogenous LTC_4. The lack of effect of dexamethasone on anaphylactic LTC_4 generation in vivo (Fig. 4, Table I) is in line with previous studies demonstrating that purified human mast cells are insensitive to the inhibitory action of dexamethasone when stimulated with anti-IgE to release LTC_4 (22). Moreover, systemic glu-

cocorticoid treatment had neither an effect on the release of LTC_4 and LTB_4, nor on the symptoms during the early-phase nasal allergic reaction after allergen challenge in humans (38, 39). A novel additional activity of glucocorticosteroids was described recently (40), showing that dexamethasone is a potent suppressor of mast cell sensitization induced by IgE antibody in a model of type I allergic inflammation in vivo. Accordingly, dexamethasone may inhibit IgE fixation to mast cells during the sensitization period, thus preventing mediator release that is induced by bridging of the receptor-bound IgE antibodies by antigen.

We did observe, however, some protection of dexamethasone-treated guinea pigs during systemic anaphylaxis as evidenced by normalization of the C_{dyn} value 60 min after OVA challenge (Fig. 5). This may be related to the inhibitory action of dexamethasone on the release of secondary mediators (41). Cysteinyl LTs are able to trigger the release of thromboxane A_2 (42, 43) and PAF (44). The LTC_4-induced release of thromboxane A_2 can be inhibited in guinea pig perfused isolated lungs by recombinant human lipocortin 1 (43). Our measurements demonstrate for the first time that dexamethasone does not act in guinea pig anaphylaxis by a suppression of LTC_4 generation. Protection against lethal anaphylactic shock has been accomplished, on the other hand, by blocking the systemic production of LTC_4 with an inhibitor of 5-lipoxygenase activation.

Summary

Leukotriene C_4 (LTC_4) underwent rapid elimination from the circulating blood and was extensively converted to LTD_4 within the vascular space of the guinea pig. To mimic the elimination and metabolism of endogenous LTC_4 generated during anaphylaxis, 14,15-^{3}H-labeled LTC_4 was infused intravenously over a period of 15 min, leading to a recovery in bile of 85% of the infused LT radioactivity within 2 h. Corresponding to the tracer studies, LTD_4 and, to a lesser extent, LTC_4 were the predominant endogenous cysteinyl LTs in guinea pig bile. The biliary production rate of endogenous LTD_4 increased from 0.3 ± 0.1 to 6.2 ± 1.8 pmol $\times$ min^{-1} $\times$ kg^{-1} ($p < 0.001$) during anaphylactic shock induced by intravenous injection of OVA (0.2 mg/kg) into sensitized guinea pigs. A novel LT biosynthesis inhibitor (MK-886; 10 mg/kg, i.v., 15 min before antigen challenge) suppressed the antigen-induced cysteinyl LT production by >92% ($p < 0.001$). This inhibition of systemic LTC_4 formation was associated with a complete protection against lethal anaphylactic shock in animals pretreated in addition with the H_1 receptor antagonist pyrilamine. Pretreatment with either the inhibitor of LT synthesis or the histamine receptor antagonist reduced the lethality during anaphylactic shock from 100 to 60 and 78%, respectively. In artificially ventilated, pyrilamine-pretreated animals, the antigen-induced decrease in dynamic lung compliance and the rise in hematocrit were significantly reduced ($p < 0.05$) by pretreatment with the inhibitor of LT synthesis. Dexamethasone at high doses (10 mg/kg, i.p., once daily for 7 d, or in a single dose of 10 mg/kg, i.v., 3.5 h before challenge) had no inhibitory effect on LT generation during anaphylaxis in vivo. However, in resident peritoneal macrophages, harvested from these dexamethasone-treated sensitized guinea pigs and stimulated with zymosan, both cysteinyl LT and 6-keto-$PGF_{1\alpha}$ formation were strongly suppressed. These studies indicate an important role of cysteinyl LTs in systemic anaphylaxis in vivo

and demonstrate the blockade of anaphylactic LT generation by a novel inhibitor of LT biosynthesis (MK-886) but not by dexamethasone.

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