Cysteinyl leukotrienes in the bile of patients with obstructive jaundice

MASAHITO UEMURA1,2, HIDEYUKI KOJIMA2, ULRIKE BUCHHOLZ1, EIRYO KIKUCHI2, MASAMI MATSUMOTO2, MASAJI KIKUKAWA2, HIROO IMAZU2, HIROSHI FUKUI2, TADASU TSUJI2, and DIETRICH KEPPLER3

1 Deutsches Krebsforschungszentrum, Heidelberg, Germany
2 Third Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara 634-8521, Japan

Background. Cysteinyl leukotrienes (LTs) are potent proinflammatory mediators. They are predominantly excreted from blood by hepatobiliary elimination. To explore the clinical significance of biliary cysteinyl LTs, we determined their concentration changes in bile during treatment in patients with obstructive jaundice.

Methods. Bile samples were obtained during endoscopic or transhepatic biliary drainage. Leukotrienes C4, D4, and E4 were quantified by two-step reversed-phase high-performance liquid chromatography and subsequent radioimmunoassay. Results. The increased excretion of cysteinyl LTs (LTC4 + LTD4 + LTE4) decreased between day 1 and 14 after drainage (means, 171 pmol/h to 79 pmol/h; P < 0.02). During drainage, the excretion was higher when there was additional cholangitis (mean, 225 and 86 pmol/h, with and without cholangitis, respectively; P < 0.001). The concentrations of LTD4 and LTE4 were also higher with additional cholangitis than without (LTD4, mean 6.0 vs 2.0 nM; P < 0.05; LTE4, 6.8 vs 2.4 nM; P < 0.02, respectively). Biliary LTC4 was detected only in patients with cholangitis. The biliary excretion of cysteinyl LTs was positively correlated with leukocyte concentration (r = 0.68; P < 0.005) and C-reactive protein (r = 0.73; P < 0.005) in blood. Furthermore, only in the absence of cholangitis, the excretion was positively correlated with serum γ-glutamyl transference (r = 0.76; P < 0.02) and alanine aminotransferase (r = 0.72; P < 0.02). Conclusions. The increased excretion of biologically active cysteinyl LTs may contribute to the aggravation of cholestasis and inflammatory reaction in obstructive jaundice.

Key words: cysteinyl leukotrienes, bile, urine, obstructive jaundice, cholangitis

Introduction

The cysteinyl leukotrienes (LTs), LTC4, LTD4, LTE4, and N-acetyl-LTE4, exert a variety of biological actions, including smooth muscle contraction and enhanced capillary leakage.1–3 Cysteinyl LTs may induce shock symptoms4–6 and may contribute to cholestasis.7,8 The liver represents the main organ responsible for the rapid uptake, metabolic inactivation, and hepatobiliary elimination of cysteinyl LTs.6–12 Hepatobiliary elimination of cysteinyl LTs predominates over renal excretion in all species. In primates, biliary excretion of cysteinyl LTs represents twice as much as urinary excretion.11–16 However, under conditions of impaired hepatobiliary elimination of cysteinyl LTs, urinary excretion of cysteinyl LTs becomes predominant over hepatobiliary excretion. We have previously demonstrated that, in patients with obstructive jaundice, the excretion of urinary cysteinyl LTs before biliary drainage increased three to four times above that in normal subjects, indicating the diversion of cysteinyl LTs from the liver to kidney in vivo.17 Alternatively, in tracer studies after the intravenous administration of [3H]LTC4 in human and monkey, 30%–40% of the radioactivity was recovered in bile within 24 h.11–16 LTE4 and LTD4 are major LTC4 metabolites in primates.11,13,15 Bile, therefore, appears as the optimal body fluid for measurement of the systemic production of cysteinyl LTs.6,10–12,15 Little information has been available previously on the analysis of cysteinyl LTs in human bile, probably because of the
difficulty entailed in purifying human bile sufficiently. Huber et al. measured the concentration of endogenous LTD₄ and LTE₄ as between 0.2 and 0.9 nM in bile samples obtained during cholecystectomy. In view of the metabolism and biological actions of cysteinyl LTs, it is of interest to determine the biliary cysteinyl LTs in patients with obstructive jaundice whose hepatobiliary elimination of cysteinyl LTs is strongly impaired. Recently, increased biliary secretion of cysteinyl LTs has been demonstrated in patients with obstructive jaundice, but information was lacking on the relationship of biliary cysteinyl LTs to clinical features, including bacterial cholangitis, which is the most frequent and critical complication associated with endotoxemia in bile duct obstruction.

The aim of this study was to quantify the endogenous cysteinyl LTs in human bile by two-step reversed-phase high-performance liquid chromatography (RP-HPLC) and subsequent radioimmunoassay (RIA), and to explore the significance of biliary cysteinyl LTs associated with clinical features and laboratory findings in patients with obstructive jaundice.

Patients and methods

Patients

The study was carried out in eight patients (six men and two women) with obstructive jaundice, aged 19 to 86 years (mean, 58 years; Table 1). Patients with asthma; renal, cardiovascular, or respiratory disease; acute extrahepatic inflammation, including urinary tract infection; gastrointestinal bleeding; or hepatocellular carcinoma were excluded from the study. No patient was positive for hepatitis B surface antigen, or antibody to hepatitis C virus. Obstructive jaundice was due to neoplasma in 20%, choledocholithiasis in 40%, and due to an injury of papilla of Vater after accident in one patient. A patient (no. 8) with obstructive jaundice did not suffer from chronic liver diseases, portal vein thrombosis, or intrahepatic or renal metastasis. The diagnosis was done at the occurrence of cholangitis on the 14th day after drainage. The patients with obstructive jaundice did not suffer from chronic liver diseases, portal ven thrombosis, or intrahepatic or renal metastasis.

Chemicals

4-Hydroxy-2,2,6,6-tetramethylpiperidine-N(1)-oxyl (HTMP) was obtained from Sigma Chemical (St. Louis, MO, USA). LTC₄, LTD₄, and LTE₄ were purchased from Cascade Biochem (University of Reading, England). [H]LTC₄ (4.8 TBq/mmol), [H]LTD₄ (1.5 TBq/mmol), and [H]LTE₄ (4.8 TBq/mm) were from Du Pont-New England Nuclear (Boston, MA, USA). N-acetyl-LTE₄ and N-acetyl-[H]LTE₄ were synthesized from LTE₄ and [H]LTE₄, respectively, as described previously. XAD-2 was obtained from Serva (Heidelberg, FRG). RP-HPLC was used to control the purity of LTs and to purify LTC₄, LTD₄, LTE₄ and N-acetyl-LTE₄ employed as standards for RIA.

Bile and urine collection and leukotriene extraction

Bile and urine were sampled simultaneously for the same 4-h time period, between 8 a.m. and 12 a.m., into bags kept below 4°C on ice. Bile was collected into ice-cold solution consisting of 90% methanol, 10 mM HTMP, and 5 mM ethylenediaminetetraacetate (EDTA) through the drainage tubes. A urine aliquot (40 ml) was mixed with 1 ml of 90% aqueous methanol (pH 8.5) containing 0.5 mM EDTA, 1 mM HTMP, and 20 mM KHCO₃. Samples collected were stored at −80°C under argon. Directly before analysis, the samples were brought to room temperature. For the analysis of LTs in bile, two samples (3 ml each) were taken; one was for both LTD₄ and LTE₄ and one for LTC₄, respectively. Each 5000 disintegrations per min (dpm) radiolabeled tracer of LTD₄ and LTE₄ was added to the same sample, and 5000 dpm radiolabeled tracer of LTC₄ was done in another sample. The samples were acidified to pH 4.0 to 4.5 with 1 M HCl, and were applied to a XAD-2 column. The column was washed with 50 ml distilled H₂O, and LTs were eluted with 15 to 20 ml of 100% aqueous ethanol containing 0.5% ammonium hydroxide. The eluates were evaporated to dryness under reduced pressure and resuspended in 30% ice-cold aqueous methanol for HPLC separation. For the analysis of LTE₄ and N-acetyl-LTE₄ in urine, one aliquot (20 ml) was taken. Each 5000 dpm radiolabeled tracer of LTE₄ and N-acetyl-LTE₄ was added to the same sample, which was acidified, centrifuged, and purified by Sep-Pak C₁₈ cartridges (Water, Milford, MA, USA) for further HPLC separation, as described earlier.

Bile samples were collected on 1 day (15 to 20 h), not immediately after drainage, in order to avoid