Hepatic uptake and metabolic disposition of leukotriene B₄ in rats

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1. In isolated perfused rat liver and *in vivo*, up to 25 % of [³H]leukotriene B₄ was eliminated from the circulation via hepatic uptake and biliary excretion within 1 h. Total body recovery of ³H amounted to about 60 % of infused [³H]leukotriene B₄. 2. Hepatobiliary excretion of leukotriene B₄ and its metabolites exceeded renal elimination by about 4-fold and depended, in contrast with excretion of cysteinyl leukotriene E₄, upon continuous taurocholate supply. 3. Analyses of bile, liver and recirculated perfusate using h.p.l.c. indicated that the liver metabolized leukotriene B₄ extensively to ω -carboxyleukotriene B₄ and its β -oxidized derivatives, and no unmetabolized leukotriene B₄ appeared in bile. These results substantiate the important contribution of the hepatobiliary system with respect to the metabolic fate of leukotriene B₄.

INTRODUCTION

Leukotriene B₄ (LTB₄) is a dihydroxylated eicosanoid which exhibits most potently pro-inflammatory effects such as chemotaxis, chemokinesis, adhesion and degranulation of phagocytic cells [1,2]. This arachidonate metabolite is formed upon stimulation of various cell types [1,3], including hepatic Kupffer cells [4,5]. Metabolism of LTB₄ as demonstrated in isolated cells occurs via ω -oxidation [6,7] by a specific cytochrome P-450-dependent 20-mono-oxygenase (EC 1.14.13.30) [8–10] and proceeds via the ω -aldehyde [11] to ω -carboxy-LTB₄ and its derivatives formed by β -oxidation from the ω -end [12]; in addition, 19-hydroxy-LTB₄ formation has been shown [12]. Another pathway of LTB₄ metabolism via initial reduction yields dihydro and dihydro-oxo derivatives [13,14].

There is ample information on the metabolism and systemic elimination of cysteinyl LTs *in vivo*, which occurs predominantly via the hepatobiliary system [15–28]. In contrast, corresponding evidence on the metabolic fate of LTB₄ *in vivo* is scarce [29], and the impact of any hepatobiliary contribution to LTB₄ elimination is unknown. The aim of this study was therefore to monitor the disposition and metabolism of circulating LTB₄ in rats *in vivo* and in the isolated perfused rat liver in order to assess the role of the hepatobiliary system in LTB₄ deactivation and elimination.

MATERIALS AND METHODS

Animals and materials

Male Wistar rats (200–250 g) were obtained from the Zentralinstitut für Versuchstiere (Hannover, Germany) and kept as described in accordance with the NIH guidelines for the use of experimental animals [30]. [5,6,8,9,11,12,14,15-³H]₈LTB₄ (7 × 10¹⁵ Bq/mol), [14,15-³H]₂LTB₄ (1.5 × 10¹⁵ Bq/mol), and [14,15-³H]₂leukotriene E₄ (LTE₄; 1.5 × 10¹⁵ Bq/mol) were purchased from New England Nuclear/DuPont (Boston, MA, U.S.A.), and sodium [¹⁴C]taurocholate (2 × 10¹² Bq/mol) was from Amersham International (Little Chalfont, Bucks., U.K.). ω -Hydroxy-LTB₄, ω -carboxy-LTB₄ and LTB₄ were obtained from Paesel (Frankfurt, Germany). Reverse-phase h.p.l.c. separation [31] was used to purify and to control purity of all LTs. Ketamine (Ketanest) was from Parke–Davis (Freiburg, Germany), xylazine (Rompun) from Bayer (Leverkusen, Germany) and 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (HTMP) from Sigma (St.

Louis, MO, U.S.A.). All other chemicals were of reagent grade and from commercial sources.

Animal experiments and liver perfusion

The animals were anaesthetized by intraperitoneal injection of ketamine (80 mg/kg body wt.) and xylazine (12 mg/kg body wt.), and the common bile duct was cannulated [16]. In experiments using whole animals, the ureters were cannulated with polyethylene tubing (inner diameter = 0.3 mm). Perfusions of isolated livers were performed in antegrade direction (via the portal vein) in a recirculating mode with erythrocyte-free medium after perfusing the liver essentially free of erythrocytes with about 600 ml of non-recirculated perfusion medium as reported [32]. In perfusion experiments studying the first-pass extraction capacity of the liver, the perfusions were performed in a non-recirculating mode for the initial 10 min time period after the onset of the 5 min tracer infusion, before switching to a recirculating mode. Sodium taurocholate (15 μ mol/h) was infused continuously into the liver perfusion medium where indicated. Bile and urine were both collected continuously under argon into ice-cold 90 % (v/v) aqueous methanol containing 1 mM-HTMP/0.5 mM-EDTA, pH 7.4. After solvent evaporation, the appropriate [³H]LTs with or without [¹⁴C]taurocholate were dissolved in 0.9 % NaCl containing 0.1 % (w/v) BSA and infused at the indicated dosages into the portal vein (liver perfusions) [32] and or into the tail vein (whole animals). At the end of the experimental time period, liver and perfusion media were sampled and deproteinized as described [33]; in whole animal experiments, aortic blood and organ samples were collected and deproteinized as reported [21].

Radiodetection and h.p.l.c. separation of LTs

Portions of the deproteinized methanolic organ and fluid samples were counted for radioactivity. The deproteinized supernatants were evaporated to dryness using a SpeedVac rotary evaporator (Savant, Farmingdale, NY, U.S.A.), resuspended in 30 % (v/v) aqueous methanol and filtered (Millex HV₄; Millipore/Waters, Milford, MA, U.S.A.) prior to reverse-phase h.p.l.c. separation. The radioactivity found remaining in these evaporated and resuspended samples was regarded as non-volatile, whereas volatile radioactive metabolites were defined as those which could be removed by the evaporation step. Separation of LTs by reverse-phase h.p.l.c. was performed on a C₁₈-

Abbreviations used: HTMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl; LT, leukotriene.

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Hypersil column (4.6 mm × 250 mm, 5 µm particles; Shandon, Runcorn, Cheshire, U.K.) with a C₁₈-precursor (Millipore/Waters). The mobile phase gradient consisted of 0.1% (v/v) acetic acid/1 mM-EDTA, pH 5.0 (adjusted with ammonium hydroxide) containing at time zero no methanol, switching at 5 min to 40% (v/v) methanol and reaching 80% (v/v) methanol at 40 min [33]. The flow rate was 1 ml/min. Continuous determination of radioactivity in the h.p.l.c. eluent was performed with a liquid scintillation device (LB 506; Berthold, Wildbad, Germany) using Rialuma scintillation mixture (Baker Chemicals, Gross-Gerau, Germany).

RESULTS

Uptake and biliary excretion of LTB₄ in isolated perfused rat liver

Livers perfused in a recirculating mode without constant infusion of exogenous taurocholate extracted only about 20% of the administered [³H]₂LTB₄ or [³H]₈LTB₄ within 1 h, about 10% of which was excreted into bile (Table 1). Most of the infused LT radioactivity was found in the perfusate after this experimental time period, and the liver contained about 10% of the infused LT radioactivity (Table 1). Constant infusion of taurocholate at a rate which maintains good bile flow throughout the liver perfusion resulted in an increased biliary excretion of radioactivity, amounting to about 20% of the infused [³H]LTB₄ dose within 1 h (Table 1). In contrast, 71 ± 2% (mean ± S.D., *n* = 3) of radioactivity from [³H]₂LTE₄ infusion was eliminated into bile without constant taurocholate administration. Separate experiments on hepatic first-pass extraction of [³H]₈LTB₄ were performed, in which total liver effluent was collected within 10 min of onset of LT infusion; under these conditions, 62 ± 11% (mean ± S.D., *n* = 3) of the infused LTB₄ escaped first-pass extraction by the liver. In contrast with the small extent of biliary ³H excretion after [³H]₈LTB₄ infusion (Table 1, Fig. 1), simultaneously administered [¹⁴C]taurocholate was quantitatively recovered in bile from such perfused livers within 40 min (Fig. 1).

Elimination and body distribution of LT radioactivity after intravenous injection of [³H]₈LTB₄ or [³H]₂LTE₄

Hepatobiliary excretion represents the major route of elimination *in vivo* of circulating cysteinyl LTs [19,22,31,34,35], including LTE₄ (Table 2). In contrast, only about 25% of LTB₄ was extracted from circulation by the hepatobiliary system in rats, of which about 22% (of the infused dose) was excreted into

bile within 1 h (Table 2). The low biliary excretion of LTB₄ in rats was not accompanied by a high renal elimination into urine (Table 2), and contents of ³H 1 h after [³H]₈LTB₄ administration were only slightly higher in liver, urine, blood and intestinal fat than after [³H]₂LTE₄ injection (Table 2). In comparison with ³H

Fig. 1. Hepatobiliary excretion of radioactivity after infusion of [³H]₈LTB₄ and [¹⁴C]taurocholate in isolated perfused rat liver

Bile was sampled continuously from perfused livers during and after combined infusion of [³H]₈LTB₄ (●, 296 kBq/kg body wt.) and [¹⁴C]taurocholate (○, 74 kBq/kg body wt.) within 5 min. Mean values (± S.D. for ¹⁴C and ± S.E.M. for ³H) from at least three perfusions/group indicate biliary radioactivity excreted within the preceding 10 min time period.

Table 2. Body distribution of [³H]₈LTB₄ and [³H]₂LTE₄ and their metabolites in rats 1 h after LT administration

Bile and urine were sampled continuously for 1 h after intravenous bolus injection of [³H]₈LTB₄ or [³H]₂LTE₄ (148 kBq/kg body wt. each). Aortic blood was withdrawn at the end of the experiment, and organs were excised and extracted for LT radioactivity [21]. Values indicate percentage of administered radioactivity (means ± S.D.) excreted within 1 h into bile and urine or remaining after 1 h in liver, kidneys, musculature, blood and intestinal fat. Asterisks indicate significant differences (**P* < 0.05, ***P* < 0.01) according to the Student's *t* test.

Table 1. Distribution of radioactivity 1 h after infusion of [³H]LTB₄ in isolated perfused rat liver

Bile was collected continuously from perfused livers during and after infusion of [³H]₈LTB₄ or [³H]₂LTB₄ (148 kBq/kg body wt.) within 5 min; liver and perfusate were sampled 1 h after the onset of LT infusion. Data correspond to the percentage of infused radioactivity recovered within 1 h in bile or detected at 1 h in liver and recirculating perfusate. Mean values ± S.D. from 3–6 perfused livers/group are given. * Without taurocholate infusion; † continuous taurocholate infusion (15 µmol/h). ‡ *P* < 0.01 as compared with biliary ³H after [³H]LTB₄ infusions without taurocholate (Student's *t* test).

Infused LT...	³ H radioactivity (% of infused dose)		
	[³ H] ₂ LTB ₄ *	[³ H] ₈ LTB ₄ *	[³ H] ₈ LTB ₄ †
Bile	9 ± 2	10 ± 1	18 ± 4‡
Liver	8 ± 1	11 ± 3	8 ± 1
Perfusate	73 ± 1	69 ± 3	70 ± 4

Infused LT...	³ H radioactivity (% of infused dose)	
	[³ H] ₈ LTB ₄ (<i>n</i> = 3)	[³ H] ₂ LTE ₄ (<i>n</i> = 4)
Bile	21.6 ± 1.0	75.0 ± 7.8**
Liver	3.6 ± 0.2	2.0 ± 0.7**
Urine	4.6 ± 1.4	1.3 ± 0.5**
Kidneys	2.3 ± 0.3	2.0 ± 0.7
Musculature	12.5 ± 0.4	8.0 ± 5.0
Blood	6.9 ± 1.4	2.5 ± 2.1*
Intestinal fat	4.1 ± 0.9	1.2 ± 0.1**
Total recovery	55.7 ± 2.7	90.0 ± 5.0**

Fig. 2. H.p.l.c. analyses of bile and liver after infusion of [³H₈]LTB₄ in isolated perfused rat liver

Bile was collected within 10–20 min (a) and liver was sampled at 60 min (b) after the onset of a 5 min [³H₈]LTB₄ infusion (148 kBq/kg body wt.) via the portal vein catheter. Following deproteinization and concentration, the appropriate non-volatile [³H₈]LTB₄ metabolites were separated and detected as described in the Materials and methods section.

from [³H₂]LTE₄, the total recovery of radioactivity in the examined body compartments after [³H₈]LTB₄ injection was significantly lower (Table 2), probably due to more extensive catabolism yielding volatile products which thus escaped our determinations.

Metabolism of LTB₄ in isolated perfused rat liver

Whereas all radioactivity in the first-pass venous effluent from liver after [³H₈]LTB₄ infusion consisted of unmetabolized LTB₄, about 90 % of the radioactivity found at 1 h in the recirculating perfusate and in liver consisted of volatile LTB₄ derivatives; about 80 % of biliary ³H, however, was non-volatile. This non-volatile ³H in bile sampled 10–20 min after LT infusion comprised ω-carboxy-LTB₄ and metabolites with h.p.l.c. retention times of 35, 32 and 21 min (Fig. 2a); these latter metabolites, including the more polar constituents in liver (Fig. 2b), probably correspond to β-oxidation products of ω-carboxy-LTB₄ some of which were identified recently [10]. Neither LTB₄ nor ω-hydroxy-LTB₄ were detectable in bile or liver (Fig. 2). Control perfusion experiments without livers indicated that [³H₈]LTB₄ remained unmetabolized and was quantitatively recovered in the perfusate after circulating in the perfusion system for 1 h (results not shown).

DISCUSSION

Our study demonstrates that, in rats, LTB₄ is extracted from the circulation by the liver to a much smaller extent (Tables 1 and 2, Fig. 1) than cysteinyl LTs (Table 2) [32,33,36], whereas the

rapid hepatic uptake and complete biliary excretion of taurocholate indicated normal liver function under these conditions (Fig. 1). In addition, the hepatobiliary excretion of LTB₄ and its metabolites was increased by choleretic bile salts (Table 1), whereas under our experimental conditions cysteinyl LT elimination into bile from isolated perfused liver [32,33] proved to be independent of such continuous taurocholate infusion. These differences in hepatic uptake of cysteinyl LTs and LTB₄ are in line with the earlier finding that LTB₄ does not compete with cysteinyl LTs for hepatocellular uptake [37]. Interestingly, the hepatobiliary excretion of LTB₄ and its metabolites exceeded renal elimination (Table 2), as it does with cysteinyl LTs [19,22,31,34,35]. On the other hand, the small extent of hepatic LTB₄ extraction (Fig. 1; Tables 1 and 2) corresponds to the minor responsiveness of the liver to this LT [38,39] as compared with the respective effects of cysteinyl LTs [33,38–41].

This investigation shows that before its partial biliary excretion (Fig. 1), LTB₄ undergoes extensive intrahepatic metabolism via ω-oxidation and β-oxidation from the ω-end (Fig. 2), as described in studies using isolated hepatocytes, liver microsomes and cytosol [10,12,42–47]. The extensive hepatocellular catabolism of LTB₄, which proceeds faster than the comparable catabolism of cysteinyl LTs [43,45], is probably responsible for the lower total recovery of radioactivity from infused [³H₈]LTB₄ (Table 2). In contrast with LTC₄ [33], however, LTB₄ remained essentially unmetabolized during its initial passage through the liver vasculature, whereas after hepatocellular uptake during repeated passages within 1 h, the liver had degraded it to ω- and β-oxidized (Fig. 2), mainly volatile, derivatives. Thus the liver exerts important functions with regard to uptake, production, inactivation and elimination not only of cysteinyl LTs, but also of the potent mediator LTB₄.

Our novel finding regarding the predominating biliary excretion of LTB₄ metabolites as compared with renal elimination offers further analytical possibilities. As we established previously with cysteinyl LTs [16,17,19,30,31], bile may also become the body fluid of choice to monitor the endogenous production of LTB₄ *in vivo* after appropriate stimulation. It will be of interest to measure such LTB₄ production under conditions of concomitantly impaired LT ω-oxidation [45], since this approach may allow the detection of unoxidized LTB₄ in bile by combined use of h.p.l.c. separation and radioimmunological quantification.

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REFERENCES

1. Ford-Hutchinson, A. W. (1985) Fed. Proc. Fed. Am. Soc. Exp. Biol. **44**, 25–29
2. Samuelsson, B., Dahlén, S.-E., Lindgren, J.-Å., Rouzer, C. A. & Serhan, C. N. (1987) Science **237**, 1171–1176
3. Lewis, R. A. & Austen, K. F. (1984) J. Clin. Invest. **73**, 889–897
4. Hagmann, W., Steffan, A.-M., Kirm, A. & Keppler, D. (1987) Hepatology **7**, 732–736
5. Sakagami, Y., Mizoguchi, Y., Seki, S., Kobayashi, K., Morisawa, S. & Yamamoto, S. (1989) Prostaglandins Leukotrienes Essent. Fatty Acids **36**, 125–128
6. Lindgren, J. Å., Hansson, G. & Samuelsson, B. (1981) FEBS Lett. **128**, 329–335
7. Powell, W. S. (1984) J. Biol. Chem. **259**, 3082–3089
8. Shak, S. & Goldstein, I. M. (1985) J. Clin. Invest. **76**, 1218–1228
9. Soberman, R. J., Harper, T. W., Murphy, R. C. & Austen, K. F. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 2292–2295

10. Harper, T. W., Garrity, M. J. & Murphy, R. C. (1986) *J. Biol. Chem.* **261**, 5414–5418
11. Soberman, R. J., Sutyak, J. P., Okita, R. T., Wendelborn, D. F., Roberts, L. J. & Austen, K. F. (1988) *J. Biol. Chem.* **263**, 7996–8002
12. Romano, M. C., Eckardt, R. D., Bender, P. E., Leonard, T. B., Straub, K. M. & Newton, J. F. (1987) *J. Biol. Chem.* **262**, 1590–1595
13. Kaever, V., Martin, M., Fauler, J., Marx, K.-H. & Resch, K. (1987) *Biochim. Biophys. Acta* **922**, 337–344
14. Powell, W. S. & Gravelle, F. (1989) *J. Biol. Chem.* **264**, 5364–5369
15. Appelgren, L.-E. & Hammarström, S. (1982) *J. Biol. Chem.* **257**, 531–535
16. Hagmann, W., Denzlinger, C. & Keppler, D. (1984) *Circ. Shock* **14**, 223–235
17. Denzlinger, C., Rapp, S., Hagmann, W. & Keppler, D. (1985) *Science* **230**, 330–332
18. Hammarström, S., Örning, L. & Bernström, K. (1985) *Mol. Cell. Biochem.* **69**, 7–16
19. Denzlinger, C., Guhlmann, A., Scheuber, P. H., Wilker, D., Hammer, D. K. & Keppler, D. (1986) *J. Biol. Chem.* **261**, 15601–15606
20. Verhagen, J., Bel, E. H., Kijne, G. M., Sterk, P. J., Bruynzeel, P., Veldink, G. A. & Vliegthart, J. F. G. (1987) *Biochem. Biophys. Res. Commun.* **148**, 864–868
21. Huber, M., Guhlmann, A., Jansen, P. L. M. & Keppler, D. (1987) *Hepatology* **7**, 224–228
22. Ezra, D., Foster, A., Cirino, M., Rokach, J. & Letts, L. G. (1987) *Prostaglandins* **33**, 717–725
23. Foster, A., Fitzsimmons, B., Rokach, J. & Letts, G. (1987) *Biochem. Biophys. Res. Commun.* **148**, 1237–1245
24. Hagmann, W. & Keppler, D. (1988) in *The Liver: Biology and Pathobiology*, 2nd edn. (Arias, I. M., Jakoby, W. B., Popper, H., Schachter, D. & Shafritz, D. A., eds.), pp. 793–806, Raven Press, New York
25. Delorme, D., Foster, A., Girard, Y. & Rokach, J. (1988) *Prostaglandins* **36**, 291–302
26. Örning, L., Keppler, A., Midtvedt, T. & Hammarström, S. (1988) *Prostaglandins* **35**, 493–501
27. Keppler, D., Huber, M., Baumert, T. & Guhlmann, A. (1989) *Adv. Enzyme Regul.* **28**, 307–319
28. Tagari, P., Foster, A., Delorme, D., Girard, Y. & Rokach, J. (1989) *Prostaglandins* **37**, 629–640
29. Serafin, W. E., Oates, J. A. & Hubbard, W. C. (1984) *Prostaglandins* **27**, 899–911
30. Hagmann, W., Denzlinger, C. & Keppler, D. (1985) *FEBS Lett.* **180**, 309–313
31. Hagmann, W., Denzlinger, C., Rapp, S., Weckbecker, G. & Keppler, D. (1986) *Prostaglandins* **31**, 239–251
32. Hagmann, W., Parthé, S. & Kaiser, I. (1989) *Biochem. J.* **261**, 611–616
33. Iwai, M., Hagmann, W., Keppler, D. & Jungermann, K. (1988) *Biol. Chem. Hoppe-Seyler* **369**, 1131–1136
34. Keppler, D., Hagmann, W. & Rapp, S. (1987) *Rev. Infect. Dis.* **9**, S580–S584
35. Keppler, A., Örning, L., Bernström, K. & Hammarström, S. (1987) *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5903–5907
36. Wettstein, M., Gerok, W. & Häussinger, D. (1989) *Eur. J. Biochem.* **181**, 115–124
37. Uehara, N., Ormstad, K., Örning, L. & Hammarström, S. (1983) *Biochim. Biophys. Acta* **732**, 69–74
38. Iwai, M. & Jungermann, K. (1988) *Biochem. Biophys. Res. Commun.* **151**, 283–290
39. Häussinger, D., Stehle, T. & Gerok, W. (1988) *Biol. Chem. Hoppe-Seyler* **369**, 97–107
40. Häussinger, D. & Stehle, T. (1988) *Eur. J. Biochem.* **175**, 395–403
41. Krell, H. & Dietze, E. (1989) *Hepatology* **10**, 300–305
42. Böstlering, B. & Trudell, J. R. (1983) *Biochem. Biophys. Res. Commun.* **114**, 850–854
43. Örning, L. (1987) *Eur. J. Biochem.* **170**, 77–85
44. Gotoh, Y., Sumimoto, H., Takeshige, K. & Minakami, S. (1988) *Biochim. Biophys. Acta* **960**, 342–350
45. Parthé, S. & Hagmann, W. (1990) *Eur. J. Biochem.* **187**, 119–124
46. Baumert, T., Huber, M., Mayer, D. & Keppler, D. (1989) *Eur. J. Biochem.* **182**, 223–229
47. Mukhtar, H., Khan, W. A., Bik, D. P., Das, M. & Bickers, D. R. (1989) *Xenobiotica* **19**, 151–159

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