Metabolic disposition of leukotriene B₄ (LTB₄) and oxidation-resistant analogues of LTB₄ in conscious rabbits

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1 The kinetics of leukotriene B₄ (LTB₄), after single i.v. injections of doses of 0.1 to 1 µg kg⁻¹, were investigated in conscious rabbits and compared with those of the Ω- and β-oxidation resistant bioactive analogues, 20, 20, 20-trifluoro-LTB₄ (20-F₃-LTB₄) and 3-thio-LTB₄, respectively.

2 Immunoreactive LTB₄ (IR-LTB₄) elimination was first-order, as shown by a constant systemic clearance (Cl_{LTB₄}) and a proportional increase in the area under the curve (AUC) of the plasma concentration versus time curve over the dose-range studied. Our results showed a good correlation between observed steady-state plasma concentrations (C_{ss}) of IR-LTB₄ after continuous infusion of LTB₄ and those predicted by using the mean estimated Cl_{LTB₄} of 93 ± 4 ml min⁻¹ kg⁻¹, further confirming the linearity of IR-LTB₄ elimination.

3 The half-life (t_{1/2}) or IR-LTB₄ increased from 0.47 ± 0.02 to 0.63 ± 0.04 min as a consequence of a change in the apparent volume of distribution (V_d) from 72 ± 5 to 109 ± 13 ml kg⁻¹, for the 0.1 and 1 µg kg⁻¹ doses injected, respectively.

4 Single i.v. injections of [³H]-LTB₄ (4.7 ng kg⁻¹) were administered, and the decay of plasma [³H]-LTB₄ following h.p.l.c. purification was used to estimate the kinetic parameters. The kinetic parameters of [³H]-LTB₄ were characterized by a mean systemic clearance (Cl) of 96 ± 11 ml min⁻¹ kg⁻¹, a t_{1/2} of 0.53 ± 0.03 min, and an apparent V_d of 85 ± 9 ml kg⁻¹, similar to the parameters obtained after LTB₄ boluses.

5 The disposition of LTB₄ analogues, whether resistant to Ω- or to β-oxidation *in vitro*, did not differ significantly from the disposition of the LTB₄ molecule. The half-lives of 20-F₃-LTB₄ and 3-thio-LTB₄ in the circulation were 0.52 ± 0.07 min and 0.70 ± 0.11 min, respectively.

6 In summary, our results showed that LTB₄, as well as Ω-oxidation- and β-oxidation-resistant analogues were cleared very rapidly from the rabbit circulation and indicate that *in situ*, metabolism in blood is not a rate-limiting factor for the elimination of LTB₄.

Keywords: Leukotriene B₄ (LTB₄) kinetics; oxidation-resistant LTB₄ analogues; LTB₄; conscious rabbit

Introduction

Leukotriene B₄ (LTB₄), a 5-lipoxygenase metabolite of arachidonic acid in leukocytes, stimulates phagocyte functional responses such as chemotaxis, adherence, superoxide anion synthesis and degranulation (Naccache *et al.*, 1989; Ford-Hutchinson, 1990). In support of its putative role in inflammation, increased local concentrations of LTB₄ have been observed in inflammatory conditions, such as purulent peritoneal exudates (Kikawa *et al.*, 1986), inflammatory bowel diseases (Fretland *et al.*, 1990), or psoriatic skin lesions (Brain *et al.*, 1984). Moreover, plasma LTB₄ levels may also be transiently elevated, as observed during reperfusion following hindlimb ischaemia in rodents (Goldman *et al.*, 1992) and rabbits (Welbourn *et al.*, 1990), and during anaphylaxis in guinea-pigs (Lee *et al.*, 1986).

Despite important progress in the characterization of the biological activities of LTB₄, knowledge of the mechanisms regulating its local or systemic levels *in vivo* is limited. On the one hand, it is well documented that Ω-oxidation represents the major metabolic pathway for LTB₄ in human isolated neutrophils (Nadeau *et al.*, 1984; Powell, 1984; Shak & Goldstein, 1984; 1985), although reduction may be favoured in neutrophils from other species (Powell, 1984; Powell & Gravelle, 1990). However, the contribution of these metabolic pathways to the regulation of LTB₄ levels *in vivo* is unclear. Indeed, Gresele *et al.* (1986) found reduced catabolism of LTB₄ in whole blood compared with isolated

leukocytes and Taylor & Sun (1985) showed that massive infiltration of neutrophils in the rat pleural cavity did not modify the disappearance of LTB₄ from the chest. On the other hand, rat hepatic and renal microsomal preparations possess Ω- and Ω-1 oxidation activities for LTB₄ (Newton *et al.*, 1985; Romano *et al.*, 1987), and the proposed routes of metabolism of LTB₄ in rat hepatocytes include Ω-oxidation and β-oxidation from the Ω-end of the 20-carboxy-LTB₄ metabolite as well as β-oxidation from the carboxyl end of LTB₄ (Harper *et al.*, 1986). These studies suggest that metabolism in the liver and kidney may be important in the regulation of LTB₄ levels *in vivo*. Accordingly, evaluation of the metabolic disposition of LTB₄ *in vivo* confirmed extensive β-oxidation of LTB₄ in the monkey and the rabbit (Serafin *et al.*, 1984) and intrahepatic Ω-oxidation followed by β-oxidation from the Ω-end in the rat (Hagmann & Korte, 1990), leading mainly to the production of volatile derivatives, with no intact LTB₄ detected in the bile or the urine. In the present study, we provide a detailed kinetic analysis of LTB₄ in conscious rabbits after single i.v. injections of unlabelled or labelled LTB₄ and further document the systemic disposition of Ω- and β-oxidation resistant analogues.

Methods

Animals

Male New Zealand rabbits (2.5–3.5 kg) purchased from Charles River (St. Constant, Québec, Canada) were used

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throughout the studies. They were maintained in individual cages with free access to food (Purina pellets) and water for at least 5 days before any experimental work was undertaken.

Single injection procedures

The rabbits were placed in restraining cages (Fenco, Boston, MA, U.S.A.); two catheters were installed, one in the marginal vein of an ear (PE-50; Clay Adams, Parsippany, NJ, U.S.A.) to allow the injection of LTB₄, and one in the central artery of an ear (Butterfly-21; Abbott Ireland LTD., North Chicago, IL, U.S.A.) to allow blood sampling. An infusion of 0.9% NaCl-5% glucose (74:26 v/v) was started at the rate of 23 ml h⁻¹ to compensate for fluid loss and for blood volume replacement. After a 60 min stabilization period, LTB₄ was injected i.v. at doses of 0.1, 0.5, or 1 µg kg⁻¹ in 1 ml vehicle. Multiple blood samples (1.2 ml) were withdrawn at $t = -30$ and $t = -5$ before and at $t = 0.5, 1, 1.5, 2, 3, 5, 8, 12, 16$ and 20 min after the bolus. The blood samples were collected in chilled polypropylene tubes containing potassium EDTA (3.7 mM, final concentration) and centrifuged for 10 min at 600 g at 4°C. Plasma was collected and kept at -70°C until assayed for LTB₄. The same blood-sampling intervals were used to assess the decay of LTB₄ analogues in plasma. 20-F₃-LTB₄ was injected at the dose of 0.5 µg kg⁻¹, whereas the dose of the 3-thio-LTB₄ analogue (available in limited quantity) was 0.16 µg kg⁻¹. In the study of LTB₄ radioactive tracer disposition, 0.3 µCi kg⁻¹ (4.7 ng kg⁻¹) of [14, 15-³H]-LTB₄ was injected.

Analytical methods

Plasma concentration of LTB₄ was determined by enzyme immunoassay (EIA) (Cayman Chemical Co., Ann Arbor, MI, U.S.A.) after reverse-phase h.p.l.c. purification of the samples, as described previously (Marleau *et al.*, 1993). Briefly, protein precipitation in plasma samples (0.4 ml) was accomplished by the addition of 4 vol of ice-cold acetonitrile containing prostaglandin B₂ (PGB₂, 20 ng ml⁻¹) as an internal standard. The supernatants (0.6 ml fractions) were diluted to 2 ml with water and purified by reverse-phase h.p.l.c. (Borgeat *et al.*, 1990). The h.p.l.c. fractions containing LTB₄ or LTB₄ analogues were collected and evaporated under reduced pressure using a Speed-Vac concentrator (Savant Instr., Farmingdale, NY, U.S.A.); the dry residues were dissolved with 50 µl of ethanol and diluted to 1 ml with EIA buffer (0.1 M phosphate buffer, pH 7.4, containing 0.4 M NaCl, 1 mM EDTA, 0.1% BSA and 0.01% sodium azide). The recovery of LTB₄ after extraction from blood and h.p.l.c. purification was 92 ± 10% ($n = 6$) (data not shown). A typical calibration curve for the assay of LTB₄ showed a slope factor of 1.002, an ED₅₀ of 38 pg ml⁻¹, and a minimal detectable amount of 9 pg ml⁻¹. Amounts of LTB₄ or LTB₄ analogues in plasma samples were quantified with calibration curves generated with the respective compounds. Plasma samples containing tritiated LTB₄ were deproteinized and purified by h.p.l.c. as described. Fractions were collected at 1 min intervals, and the radioactivity content was determined by liquid scintillation.

Kinetic determinations

The kinetic parameters were estimated by a nonlinear regression curve-fitting programme (PC-NONLIN) according to an open one-compartment model. The mean basal IR-LTB₄ concentration was subtracted from the IR-LTB₄ concentrations observed after the administration of exogenous LTB₄.

Chemicals

5(S),12(R)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (LTB₄) and 3-thio-LTB₄ were generous gifts from Dr Robert

Young (Merck Frost, Pointe-Claire, Québec, Canada), and 20,20,20-trifluoro-5(S), 12(R,S)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid (20-F₃-LTB₄) was obtained from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.). Stock solutions of LTB₄ and LTB₄ analogues (200 µg ml⁻¹ in ethanol) were kept at -20°C and diluted in phosphate buffered saline containing 0.01% bovine serum albumin (BSA) (vehicle) immediately before use. We obtained [14, 15-³H]-LTB₄ (specific activity 21.5 Ci mmol⁻¹) from Dupont (Boston, MA, U.S.A.) and BSA (low endotoxin) from Sigma Chemical Company (St. Louis, MO, U.S.A.).

Statistical analysis

The results are expressed as mean ± s.e.mean. Statistical analysis was carried out by one-way analysis of variance, using Dunnett's distribution table to assess statistical significance (* $P < 0.05$; ** $P < 0.01$).

Results

Kinetics of IR-LTB₄ and [³H]-LTB₄ after a single injection

Mean arterial basal IR-LTB₄ plasma concentrations in these groups of conscious rabbits were 0.18 ± 0.03 nM (60.2 ± 9.4 pg ml⁻¹) ($n = 19$). Thirty seconds after LTB₄ injections, arterial IR-LTB₄ concentrations increased to 2.3 ± 0.2 nM and 18.3 ± 3.5 nM ($n = 6$), for the 0.1 and 1.0 µg kg⁻¹ doses, respectively, and rapidly declined to basal levels. The data were analysed according to an open one-compartment model, as the semilogarithmic plots of plasma IR-LTB₄ concentrations versus time showed monoexponential declines until basal concentrations were reached (Figure 1). The kinetic parameters (Table 1) revealed a proportional increase in the area under the curve (AUC) of the plasma concentration versus the time curve function of the dose, resulting in a constant systemic clearance (Cl) over the dose-range studied. However, the apparent volume of distribution (V_d) increased

Figure 1 Time course of the plasma concentration of immunoreactive leukotriene B₄ (IR-LTB₄) after a single injection of LTB₄: 0.1 µg kg⁻¹ ($n = 6$) (□); 0.5 µg kg⁻¹ ($n = 7$) (Δ); 1 µg kg⁻¹ ($n = 6$) (○). The mean basal IR-LTB₄ concentration (0.18 ± 0.03 nM) was subtracted from all IR-LTB₄ concentrations measured. LTB₄ was purified by h.p.l.c. and assayed by ELISA. The results are expressed as mean (± s.e.mean).

Table 1 Kinetic parameters of immunoreactive leukotriene B₄ (IR-LTB₄) after a single i.v. injection of three different doses

Dose ($\mu\text{g kg}^{-1}$)	AUC ^a (pg min ml ⁻¹)	Cl _{LTB₄} ^b (ml min ⁻¹ kg ⁻¹)	V _d ^c (ml kg ⁻¹)	t _{1/2} (min)
0.1 (n = 6)	1177 ± 96 ^d	88 ± 7	72 ± 5	0.47 ± 0.02
0.5 (n = 7)	5592 ± 412	92 ± 6	84 ± 5	0.57 ± 0.05
1.0 (n = 6)	10897 ± 1452	98 ± 10	109 ± 13**	0.63 ± 0.04*

^aTotal area under the concentration-time curve from zero to infinity; ^bsystemic clearance of IR-LTB₄; ^capparent volume of distribution;

^dmean (± s.e.mean).

*P < 0.05; **P < 0.01.

significantly, from 72 ± 5 to 109 ± 13 ml kg⁻¹ for the 0.1 and 1 $\mu\text{g kg}^{-1}$ doses, respectively, which was accompanied by a 34% increase in the half-life. Nevertheless, the mean calculated systemic Cl could be used to predict steady-state plasma concentrations (C_{ss}) after different infusion rates (Table 2). In addition, plasma concentrations of LTB₄ increased proportionally with the infusion rate ($r^2 = 0.9990$), further supporting first-order elimination kinetics (Table 2). Figure 2 illustrates the radioactivity decay from plasma before and after purification of plasma [³H]-LTB₄ by h.p.l.c. Kinetic analysis of the data revealed a systemic Cl of [³H]-LTB₄ of 96 ± 11 ml min⁻¹ kg⁻¹, an apparent V_d of 85 ± 9 ml kg⁻¹, and a t_{1/2} of 0.53 ± 0.03 min (n = 3). These parameters were not significantly different from those calculated after the bolus (0.1 $\mu\text{g kg}^{-1}$ dose).

Disposition of LTB₄ analogues

The kinetic parameters estimated from the disappearance curves of immunoreactive 20-F₃-LTB₄ and 3-thio-LTB₄ (Figure 3) are summarized in Table 3. Overall, the parameters were not statistically different from those calculated after a bolus of LTB₄ (0.1 $\mu\text{g kg}^{-1}$). Both analogues were rapidly cleared from plasma, the 20-F₃-LTB₄ with a t_{1/2} of 0.52 ± 0.06 min (n = 4) and the 3-thio-LTB₄ with a half-life of 0.71 ± 0.06 min (n = 3).

Table 2 Plasma concentrations of immunoreactive leukotriene B₄ (IR-LTB₄) at steady state (C_{ss}) after a continuous infusion at different rates compared with the predicted C_{ss} calculated using the kinetic parameters of LTB₄ estimated after a single injection of LTB₄

Infusion rate (ng min ⁻¹ kg ⁻¹)	C _{ss} (observed) ^a (nM)	C _{ss} (predicted) (nM)
10	0.31 ± 0.01 ^b	0.32 ^c
25	0.68 ± 0.05	0.80
50	1.30 ± 0.10	1.60
100	2.68 ± 0.09	3.20

^aThe results showed a good correlation between the C_{ss} (observed) and the infusion rate (linear regression: 0.0191 ± 0.0266x, $r^2 = 0.9990$); ^bthe results are expressed as mean (± s.e.mean) (n = 4); ^cC_{ss} (predicted) were estimated using a mean Cl_{LTB₄} of 93 ml min⁻¹ kg⁻¹.

Discussion

Intravenous administration of LTB₄ produced a transient increase in arterial plasma concentrations of IR-LTB₄. The systemic Cl_{LTB₄} was constant over the dose-range studied, as indicated by the proportional increments in the AUC and the doses injected (Table 1). The mean calculated systemic Cl of LTB₄ of 93 ml min⁻¹ kg⁻¹, representing about 30% of the cardiac output in conscious rabbits (Woods *et al.*, 1989) was used to estimate the C_{ss} of LTB₄ following different infusion rates (Table 2). Indeed, the LTB₄ plasma concentrations actually measured under steady-state conditions (Marleau *et al.*, 1993) were very close those predicted and were highly

Figure 2 Decay of the total radioactivity in plasma (□) and of the radioactivity associated with [³H]-leukotriene B₄ (■) following h.p.l.c. purification (■) after a single i.v. injection of [³H]-LTB₄ (0.3 $\mu\text{Ci kg}^{-1}$) (results are from one experiment representative of three).**Table 3** Kinetic parameters of leukotriene B₄ (LTB₄) analogues after a single i.v. injection

Analogue	Dose ($\mu\text{g kg}^{-1}$)	Cl _{LTB₄} ^a (ml min ⁻¹ kg ⁻¹)	V _d ^b (ml kg ⁻¹)	t _{1/2} (min)
20-F ₃ -LTB ₄ (n = 5)	0.5	123 ± 22 ^c	125 ± 35	0.52 ± 0.07
3-thio-LTB ₄ (n = 4)	0.16	NC ^d	NC ^d	0.70 ± 0.11*

^aSystemic clearance of immunoreactive LTB₄; ^bapparent volume of distribution; ^cmean (± s.e.mean); ^dnot calculated.

*P < 0.05

Figure 3 Time course of the plasma concentrations of immunoreactive leukotriene B₄ (IR-LTB₄) analogues after a single i.v. injection of the 20-F₃-LTB₄ (0.5 µg kg⁻¹; n = 4) (□); or of the 3-thio-LTB₄ (0.16 µg kg⁻¹; n = 3) (Δ). LTB₄ analogues were purified by h.p.l.c. and assayed by ELISA. The results are expressed as mean (± s.e.mean).

correlated to the infusion rate (Table 2). Taken together, these observations further confirmed that removal of IR-LTB₄ from the circulation was proportional to the plasma concentrations over the dose-range studied.

Our results showed that LTB₄ was distributed in a volume close to the estimated plasma volume (82–102 ml kg⁻¹) in rabbits (Marleau *et al.*, 1989), suggesting that the distribution of LTB₄ is largely limited to the vascular compartment. The data also showed that the apparent *V*_d increased by 51%, with a 10 fold increase in the dose of LTB₄ injected (Table 1). A slight increase in the free fraction of drugs that are highly bound to plasma proteins in the vascular compartment (which is likely to be the case for a fatty acid derivative) is associated with an increase in the apparent *V*_d, but it should have little effect on the clearance of highly cleared drugs (Gibaldi & Perrier, 1982). From the present data, it cannot be ascertained whether the high and transient elevation of LTB₄ plasma concentrations (up to 100 fold over basal levels 30 s after the 1 µg kg⁻¹ bolus) were indeed associated with nonlinear binding of LTB₄ in the vascular compartment. However, the finding that the transient elevation in LTB₄ plasma concentrations after the bolus did not modify the mean arterial pressure (Marleau *et al.*, 1993) excludes a significant redistribution of LTB₄ secondary to haemodynamic changes. Whatever the causative factors underlying the increase in the apparent *V*_d with the dose, these changes were accompanied by a slight decrease in the *t*_{1/2} from 0.47 ± 0.02 to 0.63 ± 0.04 min, over the dose-range studied (Table 1).

In other experiments, tracer amounts of LTB₄ were administered as a bolus. As shown in Figure 2, about 80% of the total plasma radioactivity disappeared in the first 2 min after the injection; this decline was largely associated with the

disappearance of [³H]-LTB₄. In the subsequent minutes, the radioactivity associated with [³H]-LTB₄ continued to decline steadily, whereas the residual radioactivity in plasma was relatively stable up to 30 min after the i.v. injection. About 50% of the radioactivity present in plasma from 3 to 30 min after the bolus injection of [³H]-LTB₄ was found in the washings of the octadecylsilyl silica extraction cartridge (ahead of the h.p.l.c. column), strongly suggesting the appearance of small-molecular-weight polar metabolites, such as tritiated water, in the plasma. Very similar radioactivity profiles were observed after arresting an infusion of [³H]-LTB₄ in the monkey, with parallel initial decay of total and nonvolatile ³H-labelled metabolites. This was followed by the appearance of volatile labelled metabolites (mainly tritiated water) that accounted for more than 85% of the total radioactivity in plasma (Serafin *et al.*, 1984).

Previous studies on the metabolism of LTB₄ *in vitro* and *in vivo* (Harper *et al.*, 1986; Hagmann & Korte, 1990) have suggested that Ω- and β-oxidation-resistant analogues of LTB₄ might be protected from rapid degradation *in vivo*. Our results show that, despite its *in vitro* Ω-oxidation resistant property (Tanaka *et al.*, 1988), 20-F₃-LTB₄ is rapidly and efficiently removed from the circulation after a single i.v. injection (Figure 3, Table 3). We also studied the disposition of the β-oxidation resistant, 3-thio-analogue of LTB₄; the half-life of this analogue was only slightly prolonged to 0.7 min. Taken together, the present studies on the kinetics of LTB₄ and oxidation-resistant analogues *in vivo* indicate that LTB₄ is rapidly cleared from the circulation through an uptake mechanism and that Ω- and/or β-oxidation at the level of the vascular compartment do not have a significant impact on the elimination of LTB₄ from the rabbit circulation. Our studies also demonstrated that the oxidation-resistant analogues of LTB₄ are not likely to be useful in *in vivo* studies, as their uptakes and clearances from the rabbit circulation were similar to that of LTB₄. It is, however, likely that the hepatic and/or renal metabolism of 20-F₃-LTB₄ and 3-thio-LTB₄ differs significantly from that of LTB₄, which could not be assessed in the present study.

It is noteworthy that relatively long half-lives of LTB₄ have been observed after local administration. Indeed, following intradermal injections, the half-life of LTB₄ in skin sites was approximately 5 min (Aked & Foster, 1987), whereas the half-life of [¹⁴C]-LTB₄ from the inflamed pleural cavity in rats was 46 min (Taylor & Sun, 1985). In these studies, the local metabolism of LTB₄ did not appear to be a major route of elimination and it has been suggested that the stability of LTB₄ at inflammatory sites is the consequence of protein binding (Taylor & Sun, 1985). Similarly, studies in our laboratory have shown that plasma drastically reduces the metabolism of LTB₄ by human neutrophils (Palmanier & Borgeat, unpublished data; Nadeau *et al.*, 1984). In contrast, the very rapid disappearance of LTB₄ as well as of LTB₄ Ω- and β-oxidation-resistant analogues following their systemic administration suggests that protein binding in the vascular compartment would rather play a carrier role for the rapid delivery of LTB₄ at sites of uptake, possibly in the renal and hepatic vasculature.

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