Effect of the leukotriene receptor antagonists FPL 55712, LY 163443, and MK-571 on the elimination of cysteinyl leukotrienes in the rat

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1 Leukotriene elimination via bile and urine is an important mechanism of inactivation for these potent lipid mediators. We investigated whether the elimination of cysteinyl leukotrienes is a target for the action of leukotriene receptor antagonists.

2 Experiments were performed in male rats under deep thiopentone anaesthesia. The bile duct and the urinary bladder were cannulated. Tritium labelled leukotrienes and leukotriene receptor antagonists were given via central venous catheters. Elimination of leukotrienes produced *in vivo* was studied following stimulation of endogenous leukotriene biosynthesis by operative trauma. ³H-leukotriene metabolites were identified by h.p.l.c. analysis. Leukotrienes produced *in vivo* were measured by combined use of h.p.l.c. and RIA.

3 Under control conditions, $49 \pm 12\%$ of the injected ³H-leukotriene radioactivity was recovered in bile and $1 \pm 0.8\%$ in urine within 90 min. Operative trauma resulted in initial hepatobiliary secretion of $887 \pm 206 \text{ pmol kg}^{-1} \text{ h}^{-1}$ of the endogenous leukotriene metabolite N-acetyl leukotriene E₄ (LTE₄NAC).

4 FPL 55712 strongly inhibited hepatobiliary elimination of ³H-leukotriene radioactivity in a dose-dependent manner after i.v. injection of [³H]-LTC₄, [³H]-LTD₄ or [³H]-LTE₄, respectively. Biliary [³H]-LTD₄ was reduced most effectively. The leukotriene antagonist potently prevented biliary elimination of LTE₄NAC produced *in vivo*. Bile flow and elimination from blood into bile of [³H]-ouabain were also impaired by FPL 55712, but to a lesser extent.

5 LY 163443 reduced biliary [³H]-LTD₄ after i.v. administration of [³H]-LTD₄. However, the total elimination of ³H-leukotriene metabolites into bile was not significantly inhibited by the drug.

6 MK-571 reduced the biliary concentration of tracer after administration of ³H-leukotrienes most potently with respect to [³H]-LTD₄. In contrast, the total recovery of ³H-leukotrienes in bile tended to increase. This is explained by a drug-induced increase in bile flow.

7 Urinary elimination of ³H-leukotrienes, quantitatively less important in the rat, was not significantly influenced by the leukotriene receptor antagonists. Recovery of ³H-leukotriene radioactivity in liver and kidneys was quantitatively insignificant.

8 From our data, we conclude that leukotriene receptor antagonists have the potential to affect leukotriene elimination by a mechanism not necessarily related to receptor blockade. Inhibition of elimination by the receptor antagonists may prolong the biological half life of leukotrienes. This effect may counteract the antagonistic properties of these drugs.

Keywords: Leukotrienes; leukotriene receptor antagonists; leukotriene inactivation

Introduction

Cysteinyl leukotrienes are potent putative mediators of allergy and inflammation (Parker, 1987; Piper, 1989; Brain & Williams, 1990). Receptor blockade is regarded as a promising approach to antagonize leukotriene action. A variety of potent leukotriene receptor antagonists have been designed (Musser *et al.*, 1986; Snyder & Fleisch, 1989). There is no doubt that many leukotriene effects are mediated via receptors (Crooke *et al.*, 1989; Halushka *et al.*, 1989), but it is not known to date whether leukotriene inactivation also proceeds via receptor-mediated events. The effectiveness of leukotriene inactivation may be expected to be a significant determinant in the biological activity of these mediators. An important mechanism of leukotriene inactivation in rodents and primates is rapid elimination of cysteinyl leukotrienes from blood into bile and urine (Hagmann *et al.*, 1984; Denzlinger *et al.*, 1986; Foster *et al.*, 1987; Maltby *et al.*, 1990).

In the present study, we investigated the effect of the leukotriene receptor antagonists FPL 55712 (Sheard *et al.*, 1977), LY 163443 (Fleisch *et al.*, 1986), and MK-571 (Jones *et al.*, 1988) on the elimination of ³H-labelled cysteinyl leukotrienes into bile and urine of anaesthetized rats. In addition, the effect

of FPL 55712 on the elimination of leukotrienes generated *in vivo* was studied.

A preliminary report on minor parts of this work has been presented earlier (Denzlinger *et al.*, 1990a).

Methods

Experimental protocol

Experiments were performed in male Wistar rats (180–220 g) under deep thiopentone anaesthesia (initial dose, 75 mg kg^{-1} , i.p.). Both jugular veins were cannulated followed immediately by the infusion of physiological saline at $700 \mu\text{l h}^{-1}$. The bile duct and urinary bladder were cannulated. Body temperature was kept above 35°C by infra-red radiation.

Bile and urine were collected continuously into ice cold aqueous methanolic (90 vol.%) solution containing HTMP, 1 mmol l^{-1} , and EDTA, 0.5 mmol l^{-1} , pH adjusted to 7.4. Sampling periods were 10 min for bile and 90 min for urine. Bile flow and urine production were determined by weight.

In the tracer experiments, leukotriene receptor antagonists and their vehicles were given into one jugular vein as a continuous infusion after a bolus injection starting 3 min before administration of ³H-leukotrienes. ³H-labelled leukotrienes

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were adjusted to give a final specific activity of 12 TBq mol^{-1} , diluted in physiological saline and injected as a bolus via central venous catheter at a dose of $37 \text{ kBq per kg body weight}$, corresponding to $3 \text{ nmol per kg body weight}$. ^3H -leukotrienes were given twice: at 0 min together with the appropriate vehicle for the leukotriene receptor antagonists and at 90 min together with the drug to be tested, or again with the vehicle alone (control experiments). At the end of the experiments, 2 ml of blood were withdrawn by puncture of the aorta. The liver and kidneys were removed and immediately frozen in liquid nitrogen. Bile, urine and blood plasma in methanolic solution, as well as the frozen organs were stored at -40°C . Aliquots of the biological samples were counted for tritium radioactivity. In addition, bile was analysed by high performance liquid chromatography (h.p.l.c.) for ^3H -leukotriene metabolites.

In the experiments on elimination of leukotrienes generated in anaesthetized rats, the operative trauma necessary for sample collection (i.e. laparotomy and cannulation of the bile duct) was used as a stimulus for endogenous leukotriene generation. FPL 55712 ($20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$) or the vehicle were given as a continuous infusion starting 10 min before the laparotomy and continuing until 30 min after the operation.

High-performance liquid chromatography

Isocratic h.p.l.c. was performed as described earlier (Denzlinger *et al.*, 1986; 1990b) on a C18 Hypersil column ($4.6 \times 250 \text{ mm}$, $5 \mu\text{m}$ particles, Shandon, Runcorn, Cheshire) with a C18 precolumn (Waters, Milford, MA, U.S.A.). The mobile phase consisted of methanol, water, acetic acid (65:35:0.1 by volume), EDTA, 1 mmol l^{-1} , pH 5.6 adjusted with ammonium hydroxide. The flow rate was 1 ml min^{-1} . Bile was deproteinized for h.p.l.c. by storage in 80% aqueous methanol at -40°C for at least 12 h and centrifugation at $2500 g$ for 20 min at -10°C . Storage and centrifugation were repeated, if necessary. The supernatant was concentrated to dryness in a centrifuge under low pressure (Savant Speed Vac, Framingdale, NY, U.S.A.) and redissolved in 30% aqueous methanol. In the studies on tracer elimination, ^3H -leukotriene metabolites were identified by comigration with authentic leukotriene standards. In the studies on elimination of leukotrienes generated *in vivo*, an aliquot of h.p.l.c. fractions was counted for tritium radioactivity to determine retention times of ^3H -leukotriene standards added in trace amounts to the samples prior to h.p.l.c. Another aliquot was stored at -20°C under argon for further analysis by radioimmunoassay (RIA).

Radioimmunoassay

RIA analysis was performed in aliquots of h.p.l.c. fractions as described earlier (Denzlinger *et al.*, 1986; 1990b). The antiserum was diluted 1:5700. [^3H]-LTC₄ (130 Bq) was used as radioligand. The lower detection limit of the assay system was below 10 fmol for LTC₄, LTD₄, LTE₄ and LTE₄NAC; the relative percentage cross reactivities on a molar basis at 50% binding were 100, 68, 53 and 82, respectively. LTB₄ does not cross react in this system at amounts of up to $1 \mu\text{mol}$.

Materials

Leukotriene receptor antagonists were kindly provided by Fisons, Loughborough, Leicestershire (FPL 5571), Eli Lilly, Indianapolis, IN, U.S.A. (LY 163443), and Merck Frosst, Quebec, Canada (MK-771). FPL 55712 (sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate) was dissolved in 5% glucose. LY 163443 (1-[2-hydroxy-3-propyl-4-{4-(1H-tetrazol-5-ylmethyl)phenoxy}methyl}phenyl]ethanone) was dissolved in sodium carbonate/bicarbonate buffer, 50 mmol l^{-1} , pH 9.0 and diluted in physiological saline. MK-571 (former name L-660, 711, (3-[3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl]-(3-[dimethylamino-3-oxo propyl]

thio)methyl}thio)propanoic acid) was dissolved in physiological saline. In a guinea-pig ileum assay system $0.1 \mu\text{mol l}^{-1}$ of FPL 55712, LY 163443 or MK-571 reduced contraction induced by 8 nmol l^{-1} LTD₄ to 65, 57, or 12% of control values, respectively.

Unlabelled LTC₄, LTD₄ and LTE₄ were purchased from Paesel, Frankfurt, F.R.G. 14,15- ^3H -LTC₄, 14,15- ^3H -LTD₄ and ^3H -ouabain were obtained from Amersham International, Buckinghamshire. 14,15- ^3H -LTE₄ was bought from DuPont, Boston, MA, U.S.A. N-acetyl LTE₄ (LTE₄NAC) and ^3H -LTE₄NAC were synthesized from LTE₄ and ^3H -LTE₄ as described by Hagmann *et al.* (1986). The concentration of unlabelled leukotrienes was determined by absorbance measurements at 280 nm. 4-Hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (HTMP) was bought from Sigma, St. Louis, MO, U.S.A.

Thiopentone (Trapanal) was purchased from Byk Gulden, Konstanz, F.R.G. The rabbit cysteinyl leukotriene antiserum was kindly donated by Professor B.A. Peskar, Ruhr-Universität, Bochum, F.R.G.

Statistics

The *t* test for paired observations was used to analyse significance of differences between ^3H -leukotriene elimination or bile flow respectively during vehicle and leukotriene antagonist administration. The *t* test for unpaired observations was used to calculate significance between elimination of leukotrienes generated *in vivo* in the treated and untreated animals. The null hypothesis was rejected in $P < 0.05$.

Results

Effects of leukotriene receptor antagonists on elimination of ^3H -leukotriene radioactivity

After i.v. injection of ^3H -labelled cysteinyl leukotrienes in anaesthetized rats, $49 \pm 12\%$ (mean $\pm$ s.d., $n = 31$) of the tritium radioactivity was recovered in bile within 90 min. Only $1.0 \pm 0.8\%$ (mean $\pm$ s.d., $n = 31$) of injected tritium radioactivity was found in urine, and radioactivity in blood was below the detection limit after 90 min.

Elimination characteristics of ^3H -leukotrienes were highly reproducible if the injection was repeated in the same rat (Figure 1, vehicle). In the pharmacological studies ^3H -leukotrienes were injected first (at 0 min) together with the vehicle for the drug to be tested, second (at 90 min) together with the drug to enable intra-individual comparison of ^3H -leukotriene secretion. Elimination of ^3H -leukotrienes during administration of leukotriene antagonist was related to elimination of ^3H -leukotrienes during administration of vehicle.

Using this experimental design we obtained the following results. FPL 55712 strongly reduced hepatobiliary elimination of ^3H -leukotrienes in a dose-dependent manner (Figure 1, Table 1). Infusion of $20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ of FPL 55712 decreased elimination of [^3H]-LTC₄ to $23.1 \pm 9.8\%$ of control (mean $\pm$ s.d.). This corresponds to less than 13% of the injected radioactivity recovered in bile. Reduction of ^3H -leukotriene elimination by FPL 55712 was more pronounced with respect to the total amount of radioactivity secreted (Table 1A) than with respect to the tritium concentration in bile (Table 1C) because FPL 55712 also reduced bile flow (Table 1B).

Hepatobiliary elimination of [^3H]-ouabain was also inhibited by FPL 55712, but to a lesser extent. Using the same experimental design as for ^3H -leukotrienes, $20 \mu\text{mol kg}^{-1} \text{ h}^{-1}$ of the leukotriene antagonist reduced elimination of [^3H]-ouabain to $60.8 \pm 8.8\%$ (mean $\pm$ s.d.) of control.

LY 163443, $25 \mu\text{mol kg}^{-1} \text{ h}^{-1}$, caused a slight decrease in total ^3H -leukotriene elimination into bile that was neither sta-

Figure 1 Effect of FPL 55712 on the recovery of ^3H -leukotriene radioactivity in bile. Representative results obtained in two rats are shown. One rat ($\square$) was treated twice with the vehicle (5% glucose). In the other rat ($\bullet$) FPL 55712 was administered during and after the second injection of [^3H]-LTC $_4$. The tracer was injected at 0 and at 90 min. Intravenous bolus injections of the vehicle or FPL 55712 ($10\text{ }\mu\text{mol kg}^{-1}$) were given 3 min before the tracer, as indicated by arrows. The bolus was followed by infusion of the vehicle or FPL 55712 ($20\text{ }\mu\text{mol kg}^{-1}\text{ h}^{-1}$) as indicated by bars. Bile was sampled continuously. Percentage recoveries of the injected dose detected in the respective bile fractions are indicated.

tistically significant on the basis of the total ^3H -leukotriene recovery in bile (Table 1A) nor on the basis of total biliary ^3H -leukotriene concentrations (Table 1C). The drug also had no significant effect on bile flow (Table 1B).

MK-571, $15\text{ }\mu\text{mol kg}^{-1}\text{ h}^{-1}$, strongly reduced biliary concentrations of tritium radioactivity after i.v. injection of ^3H -leukotrienes (Table 1C). In contrast, the total amount of ^3H -leukotrienes recovered in bile tended to increase. The latter effect was statistically significant after application of ^3H -LTC $_4$ (Table 1A). The opposing actions of MK-571 on biliary ^3H -leukotriene concentrations and total elimination of tracer may in part be explained by a choleric effect of MK-571 resulting in an increase in bile flow averaging 141% of control (Table 1B).

Cysteinyl leukotriene elimination into urine, quantitatively less important in the rat, was not influenced to a statistically significant extent by the leukotriene receptor antagonists.

Under control conditions, 90 min after injection of ^3H -leukotrienes, radioactivity in blood was below the detection limit and radioactivity in liver and kidneys amounted to 0.011 or 0.003% of injected dose, respectively. At that time, none of the leukotriene receptor antagonists significantly increased the ^3H -leukotriene concentration in blood or the ^3H -leukotriene content of the kidneys. There was only a small but significant increase in ^3H -leukotriene content in the liver (0.015%) following $20\text{ }\mu\text{mol kg}^{-1}\text{ h}^{-1}$ of FPL 55712.

Effects of leukotriene receptor antagonists on the pattern of biliary ^3H -leukotriene metabolites

Bile samples obtained during the experiments described in the previous section were analysed by h.p.l.c. to evaluate the effects of the leukotriene receptor antagonists on the proportions of biliary ^3H -leukotriene metabolites i.e. [^3H]-LTC $_4$, [^3H]-LTD $_4$, [^3H]-LTE $_4$, [^3H]-LTE $_4$ NAc and polar ^3H -leukotriene metabolites (i.e. metabolites more polar than [^3H]-LTC $_4$).

Under control conditions, the metabolite pattern was similar after i.v. [^3H]-LTC $_4$ or [^3H]-LTD $_4$ apart from a greater proportion of [^3H]-LTC $_4$ in the first bile fraction after i.v. [^3H]-LTC $_4$. Proportions in bile of [^3H]-LTC $_4$ and [^3H]-LTD $_4$ decreased in a time-dependent manner as proportions

Table 1 Effects of FPL 55712, LY 163443, and MK-571 on (A) the recovery of ^3H -leukotriene radioactivity in bile, (B) bile flow, (C) the biliary concentration of ^3H -leukotriene radioactivity, and (D) the contribution of [^3H]-LTD $_4$ to biliary ^3H -leukotriene radioactivity

		FPL 55712		LY 163443	MK-571
Bolus ($\mu\text{mol kg}^{-1}$)		10		2.5	75
Infusion [$\mu\text{mol kg}^{-1}\text{ h}^{-1}$]		20		5	25
					15
(A) ^3H -leukotriene-recovery in bile					
(% of control, mean $\pm$ s.d.)	[^3H]-LTC $_4$	23.1 $\pm$ 9.8 ^c	ND	ND	110.3 $\pm$ 0.2 ^c
	[^3H]-LTD $_4$	35.3 $\pm$ 14.9 ^a	66.9 $\pm$ 1.7 ^c	95.1 $\pm$ 7.1	102.4 $\pm$ 3.0
	[^3H]-LTE $_4$	40.7 $\pm$ 16.8 ^a	ND	91.5 $\pm$ 18.4	ND
(B) Bile flow					
(% of control, mean $\pm$ s.d.)		75.8 $\pm$ 15.8 ^c	81.8 $\pm$ 10.5	97.1 $\pm$ 19.5	140.9 $\pm$ 9.9 ^c
(C) ^3H -leukotriene-concentration in bile					
(% of control, mean $\pm$ s.d.)	[^3H]-LTC $_4$	35.1 $\pm$ 16.7 ^b	ND	ND	52.0 $\pm$ 6.7 ^b
	[^3H]-LTD $_4$	57.2 $\pm$ 14.2 ^a	78.2 $\pm$ 10.6	84.2 $\pm$ 9.9	47.3 $\pm$ 13.9 ^a
	[^3H]-LTE $_4$	56.0 $\pm$ 10.9 ^a	ND	105.1 $\pm$ 35.3	ND
(D) Contribution of [^3H]-LTD $_4$ to biliary ^3H -leukotrienes					
(% of control, mean $\pm$ s.d.)		27.3 $\pm$ 14.6 ^a	ND	55.7 $\pm$ 12.2 ^a	51.7 $\pm$ 15.2 ^a

Experiments were performed as outlined in Figure 1. Leukotriene receptor antagonists were given as continuous infusion following bolus injection.

(A) The total amount of ^3H -leukotriene radioactivity recovered in bile within 90 min after the second injection of [^3H]-LTC $_4$, D $_4$ or E $_4$ during infusion with leukotriene antagonist was compared to the total amount of ^3H -leukotriene radioactivity recovered within 90 min after the first injection of tracer during infusion of the vehicle (control, 100%).

(B) Bile flow during infusion with leukotriene antagonist was compared to bile flow during the preceding control period with vehicle given alone (control, 100%). Under control conditions bile flow was $4.2 \pm 0.4\text{ }\mu\text{mol kg}^{-1}\text{ h}^{-1}$ (mean $\pm$ s.d.).

(C) The biliary concentration of ^3H -leukotriene radioactivity after the second injection of ^3H -leukotriene during infusion with leukotriene antagonist was compared to the biliary concentration of ^3H -leukotriene radioactivity after the first injection of tracer during infusion of vehicle (control, 100%).

(D) The contribution of [^3H]-LTD $_4$ to the total of biliary ^3H -leukotriene metabolites obtained 0–10 min after the second injection of [^3H]-LTD $_4$ during infusion of leukotriene antagonist was compared to the proportions within the 0–10 min interval after the first injection of tracer during infusion of vehicle (control, 100%).

Mean values $\pm$ s.d. from at least 3 animals are indicated. ^a, ^b, ^c indicate a statistically significant difference from control by $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively, as determined by the t test for paired events. ND: not determined.

Figure 2 Effect of FPL 55712 on the elimination of cysteinyl leukotrienes generated *in vivo*. Endogenous leukotriene generation was elicited by the operative trauma necessary for sample collection (laparotomy and cannulation of the bile duct) at 0 min. Intravenous infusion of FPL 55712 ($20 \mu\text{mol kg}^{-1} \text{h}^{-1}$, ●) or the vehicle (5% glucose, □) was started 10 min before laparotomy and continued until 30 min after the operation. Mean values $\pm$ s.d. of biliary elimination of LTE_4NAC from 3 rats are shown. The difference between the treated and untreated animals was statistically significant from 0 to 80 min (i.e. until 40 min after end of FPL 55712 infusion) as determined by *t* test for unpaired events.

of [^3H]- LTE_4NAC and of the polar ^3H -leukotriene metabolites increased. Only [^3H]- LTE_4NAC , polar ^3H -leukotriene metabolites, and small amounts of [^3H]- LTE_4 were detected after i.v. [^3H]- LTE_4 . Contribution of [^3H]- LTE_4 to biliary ^3H -leukotrienes never exceeded 5%. Cumulative secretion of [^3H]- LTE_4NAC was predominant under all conditions tested.

Leukotriene receptor antagonists had a significant effect on the biliary ^3H -leukotriene metabolite pattern only with respect to biliary [^3H]- LTD_4 . [^3H]- LTD_4 was the predominant biliary ^3H -leukotriene metabolite only within the first 10 min bile fraction after i.v. [^3H]- LTC_4 or [^3H]- LTD_4 . After i.v. [^3H]- LTD_4 proportions in this fraction were 6.1 ± 1.6 [^3H]- LTC_4 : 44.7 ± 11.8 [^3H]- LTD_4 : 1.1 ± 2.3 [^3H]- LTE_4 : 24.4 ± 7.5 [^3H]- LTE_4NAC : 23.8 ± 7.6 polar ^3H -leukotriene metabolites (%), mean $\pm$ s.d., $n = 9$). In this initial phase of ^3H -leukotriene elimination, the relative contribution of [^3H]- LTD_4 to the total biliary ^3H -leukotriene metabolites was strongly reduced by each of the leukotriene antagonists tested (Table 1D). During infusion with the leukotriene antagonists, the proportions of [^3H]- LTC_4 and of the polar ^3H -leukotriene metabolites also tended to decrease and the proportion of [^3H]- LTE_4NAC tended to increase. However, these changes were not statistically significant.

Effects of FPL 55712 on elimination of leukotrienes produced *in vivo*

Stimulation of endogenous leukotriene generation in anaesthetized rats by the operative trauma necessary for sample collection (laparotomy and cannulation of the bile duct) resulted in the initial hepatobiliary secretion of $887 \pm 206 \text{ pmol kg}^{-1} \text{h}^{-1}$ (mean $\pm$ s.d.) of LTE_4NAC as

detected by h.p.l.c. and RIA. The secretion rate gradually decreased within 90 min following the operative trauma (Figure 2). FPL 55712 ($20 \mu\text{mol kg}^{-1} \text{h}^{-1}$) reduced the initial secretion of LTE_4NAC to $13 \pm 6 \text{ pmol kg}^{-1} \text{h}^{-1}$ (mean $\pm$ s.d.). Secretion of LTE_4NAC slowly increased after discontinuation of FPL 55712 infusion (Figure 2). The difference between the treated and the untreated animals was statistically significant until 40 min after the antagonist infusion had been terminated. No endogenous leukotriene metabolites other than LTE_4NAC were detected in bile by h.p.l.c. and RIA analysis.

Discussion

Our results demonstrate that leukotriene receptor antagonists may affect the biological inactivation of cysteinyl leukotrienes by inhibition of their hepatobiliary elimination. Elimination characteristics of ^3H -leukotrienes (Figure 1), ^3H -leukotriene metabolites, and of leukotrienes produced *in vivo* following operative tissue trauma (Figure 2) were in agreement with results from previous investigations (Hagmann *et al.*, 1984; Denzlinger *et al.*, 1985; Hagmann *et al.*, 1986; Foster *et al.*, 1987). The efficiency of hepatobiliary clearance of leukotrienes from circulating blood allows their determination in bile as a measure for leukotrienes generated *in vivo*. This has been demonstrated in the rat following administration of lipopolysaccharide (Hagmann *et al.*, 1984), tumor necrosis factor (Huber *et al.*, 1988), platelet-activating factor (Huber & Keppler, 1987), frog virus (Hagmann *et al.*, 1987), after pulmonary anaphylaxis (Foster *et al.*, 1988) and after different kinds of tissue trauma (Denzlinger *et al.*, 1985). Urinary leukotrienes are much less indicative of endogenous leukotriene production in the rat as only a very small portion of leukotrienes is cleared into urine in this species.

In the tracer studies, leukotriene receptor antagonists were given as a continuous infusion following a bolus injection to achieve a rapid and constant saturation of binding sites. In the studies on elimination of leukotrienes generated *in vivo* a 10 min period of leukotriene antagonist infusion preceded the operative trauma used as a stimulus for endogenous leukotriene production. At the doses applied, effective leukotriene antagonistic actions of the leukotriene receptor antagonists may be expected to occur *in vivo*. FPL 55712, $20 \mu\text{mol kg}^{-1}$ repeatedly i.p. has been used to antagonize leukotriene actions in a mouse model of endotoxin shock (Hagmann *et al.*, 1984). LY 163443, $77 \mu\text{mol kg}^{-1}$ p.o. (Fleisch *et al.*, 1986) and MK-571, $1.9 \mu\text{mol kg}^{-1}$ p.o. (Jones *et al.*, 1988) have been shown to improve significantly pulmonary parameters impaired by LTD_4 *in vivo* in guinea-pigs and monkeys, respectively.

FPL 55712 was a strong inhibitor of hepatobiliary elimination of ^3H -leukotrienes (Figure 1, Table 1). This effect was partially due to reduction in bile flow induced by FPL 55712 (Table 1B) and also reflected in impaired secretion of [^3H]-ouabain. Inhibition of ^3H -leukotriene elimination, however, markedly exceeded inhibition of bile flow. The effect of FPL 55712 was most pronounced with respect to reduction of biliary [^3H]- LTD_4 . The small portion of ^3H -leukotriene radioactivity detected after 90 min in liver and kidneys does not significantly account for the radioactivity not recovered in bile and urine. The small increase in ^3H -leukotriene radioactivity in the liver after administration of FPL 55712 also does not account for the reduced biliary recovery of tracer. Most likely, leukotrienes not eliminated via bile are subjected to oxidative degradation (Örning, 1987). This process may be expected to play a major role under conditions where hepatobiliary elimination is impaired.

Our finding that FPL 55712 dramatically reduces the hepatobiliary elimination of leukotrienes produced *in vivo* (Figure 2) expands our findings from the tracer studies to a pathophysiological situation *in vivo*.

In contrast to FPL 55712, LY 163443 was only a weak inhibitor of total ^3H -leukotriene elimination in our rat

model. Only secretion of [^3H]-LTD $_4$ was significantly reduced by the drug (Table 1).

MK-571 inhibited biliary ^3H -leukotriene secretion with respect to biliary ^3H -leukotriene concentrations. [^3H]-LTD $_4$ in bile was most strongly reduced. The drug did not inhibit but rather enhanced the total biliary ^3H -leukotriene elimination (Table 1). This was apparently due to a strong choleretic effect of MK-571 compensating for reduced biliary ^3H -leukotriene concentrations induced by the drug.

Elimination of cysteinyl leukotrienes via urine was not significantly influenced by the leukotriene receptor antagonists. This question should be re-evaluated in primates where renal clearance of leukotrienes is quantitatively more important than in the rat (Denzlinger *et al.*, 1986; Maltby *et al.*, 1990).

From our data, we cannot define the mechanism of action of leukotriene receptor antagonists responsible for the effect on hepatobiliary leukotriene elimination. Knowledge concerning leukotriene elimination is very limited. The rapid time course of biliary clearance of leukotrienes from blood suggests that receptor-mediated events might be involved. If receptors were involved, one might speculate whether they were similar or different from leukotriene receptors mediating leukotriene effects. The pharmacological activity of FPL 55712, LY 163443 and MK-571 has been described to be selectively directed against LTD $_4$ /LTE $_4$ receptors (Fleisch *et al.*, 1986; Jones *et al.*, 1988; Snyder & Fleisch, 1989). Rapid conversion in circulating blood of LTC $_4$ to LTD $_4$ and LTE $_4$ (Huber & Keppler, 1987) may explain why the principal elimination characteristics of i.v. injected [^3H]-LTC $_4$, [^3H]-LTD $_4$, and [^3H]-LTE $_4$ were similar under control conditions. Only the contribution of [^3H]-LTC $_4$ and [^3H]-LTD $_4$ to the ^3H -leukotriene metabolite pattern in the early bile fractions depended on the administered ^3H -leukotriene metabolite. The similarity in elimination characteristics of ^3H -leukotriene metabolites appeared to be largely preserved in the presence of the leukotriene receptor antagonists except for [^3H]-LTD $_4$, the concentration of which in bile was preferentially reduced by the drugs (Table 1D). Preferential or even exclusive reduction of biliary [^3H]-LTD $_4$ by FPL 55712, MK-571, or LY 163443 respectively, could be in line with a specific receptor component in leukotriene elimination impaired by LTD $_4$ antagonism. The relative potency of the leukotriene receptor antagonists used in inhibiting leukotriene actions in organ preparations *in vitro* (e.g. guinea-pig ileum assay, MK-571 > LY 163443 > FPL 55712) is, however, not related to their relative impact on ^3H -leukotriene elimination (Table 1).

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This argues in favour of the interpretation that receptors mediating leukotriene actions and, if existent, receptors mediating leukotriene elimination may be different.

Recently, almost complete biliary secretion and pronounced enterohepatic circulation of a LTD $_4$ /E $_4$ antagonist has been demonstrated (Christensen *et al.*, 1990). This raises the possibility that leukotrienes and leukotriene receptor antagonists might compete for a common transport system.

In conclusion, our data show that leukotriene inactivation by hepatobiliary elimination is a potential target for leukotriene receptor antagonists. Inhibition of leukotriene elimination by receptor antagonists may prolong the biological half life of leukotrienes. This effect may counteract the leukotriene antagonistic properties of these drugs *in vivo*. Our data do not predict that the demonstrated effects of the leukotriene receptor antagonists are of relevance for the therapeutic situation *in vivo*. Doses required for therapeutic use may be lower than those used in the present study. It is possible that in primates alternative mechanisms of leukotriene inactivation (renal elimination and oxidative degradation) are sufficiently active to compensate for impaired hepatobiliary elimination. Even if enhanced local or systemic leukotriene concentrations should occur, they might be too small to cause any significant effect, especially in the presence of a leukotriene receptor antagonist. It is suggested, however, that leukotriene antagonists should be screened for their effect on leukotriene inactivation. Leukotriene receptor antagonists with minor effects on leukotriene elimination (LY 163443) or inducing effects compensatory to their inhibitory action of leukotriene elimination (MK-571) seem more suitable to antagonize leukotriene effects *in vivo* than receptor antagonists with a strong inhibitory effect on leukotriene inactivation (FPL 55712 in the rat model used). On the other hand, elucidation of the way in which leukotriene receptor antagonists affect elimination of leukotrienes from blood into bile will help define the mechanisms underlying hepatobiliary secretions of these eicosanoids. This may in turn be of relevance for the future development of leukotriene antagonistic drugs.

We are indebted to Prof. B.A. Peskar, Bochum, F.R.G., for providing the leukotriene antibody. We wish to thank Prof. R. Schulz, München, under whose guidance the guinea-pig ileum assays with the leukotriene receptor antagonists were performed. We are grateful for the leukotriene receptor antagonists generously provided by Fisons, Eli Lilly and Merck Frosst. This work was supported by the Deutsche Forschungsgemeinschaft (De 397/1-2).

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(Received September 17, 1990

Revised December 3, 1990

Accepted December 17, 1990)