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DIFFERENT PATHOMECHANISMS OF ALTERED BILIARY LEUKOTRIENE C_4 ELIMINATION IN ISOLATED PERFUSED RAT LIVERS

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Abstract—Hepatic retention of cysteinyl leukotrienes is a consequence of impaired bile secretion and may be involved in the pathogenesis of intrahepatic cholestasis. In order to assess the mechanisms of altered biliary leukotriene elimination, we studied the secretion and metabolic pattern of leukotriene C_4 (LTC_4) in bile early in the alterations of bile formation by xenobiotics. To this end, rats were pretreated with α-naphthylisothiocyanate (ANIT), ethionine (ETH), or estradiol valerate (EV) at doses which did not increase serum marker enzymes of cholestasis. Bile secretion was assessed in perfused livers isolated from the treated rats. In all models, the access of [^{14}C]sucrose into bile was increased, indicating increased permeability of the bile tract. Biliary recovery of radioactivity infused as [^{3}H]LTC_4 was decreased by ANIT and ETH while ^{3}H-efflux into the perfusate was increased concomitantly. The secretion rate of ^{3}H-radioactivity into bile was correlated with that of [^{14}C]taurocholate infused at the same time. After pretreatment with ANIT (but not in the other models) the venous efflux of [^{3}H]LTC_4-derived radioactivity was correlated with the access of [^{14}C]sucrose into bile. Accordingly, only after ANIT pretreatment was increased [^{14}C]sucrose clearance into bile associated with greatly enhanced biliary access of [^{32}P]phosphate. Thus, altered charge selectivity of the paracellular pathway appears to be a prerequisite for reflux of cholephilic anions. HPLC analysis of [^{3}H]LTC_4-derived radioactivity in bile revealed that in all models of altered bile secretion the relative amount of LTD_4 in bile was elevated. These results demonstrate differential changes in hepatobiliary transport and metabolism of LTC_4 in developing cholestasis. ANIT inhibits leukotriene secretion by increasing paracellular permeability with loss of charge selectivity. In contrast, ETH treatment inhibits transcellular transport while treatment with EV only results in enhanced LTC_4 metabolism.

Key words: paracellular permeability; cholestasis; cysteinyl-leukotrienes; α-naphthylisothiocyanate; ethionine; estradiol valerate

Leukotrienes (LTs§) are arachidonic acid metabolites resulting from the primary catalytic action of arachidonate 5-lipoxygenase (EC 1.13.11.34.) [for reviews see Refs. 1–7]. These compounds are potent mediators of inflammation, being released mainly from monocytes, macrophages, mast cells and polymorphonuclear leukocytes [8]. The epoxide leukotriene A_4 can be hydrolysed to LTB_4 or conjugated with glutathione to form LTC_4 [4, 5, 9–13]. Degradation of the glutathione moiety results in LTD_4 and LTE_4 by the catalytic action of γ-glutamyltransferase (EC 2.3.2.2.) and dipeptidase (EC 3.4.13.11.). As for the main physiological functions, LTB_4 is a potent chemotactic agent [14] whereas the cysteinyl LTs (LTC_4, LTD_4 and LTE_4) induce smooth muscle contraction and increase vascular permeability [15]. Hepatocytes are the most important site of elimination where leukotrienes are taken up, degraded and secreted into bile [12, 13, 16–18]. An active, carrier-mediated transport process across the canalicular membrane was shown for cysteinyl LTs [19, 20].

It was previously suggested that cysteinyl LTs are mediators of liver injury [12, 13, 21, 22]. An increase in LT secretion into the bile of rats was observed after tissue trauma [23] and in endotoxin-mediated shock [24, 25].

Intrahepatic cholestasis is an injury of the liver that is often associated with hepatic inflammation. It can be caused by numerous chemicals and is a side effect of certain drugs [26–28]. At the organ level, we demonstrated different primary mechanisms of inhibition of bile secretion [29] including impairment of bile fluid formation [30], bilio-sinusoidal reflux due to increased permeability of the bile tract [31, 32], and reflux of cholephilic compounds from the cytosol due to changed equilibria across the sinusoidal membrane of hepatocytes [33]. In the models of primarily increased permeability of the bile tract this alteration preceded hepatocellular enzyme release indicating minimal changes [31, 32]. Thus, modified bilio-sinusoidal distribution of cholephilic compounds such as LTs may be a pathogenetic factor in these early stages of liver injury.

Therefore, in order to study interactions between

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§ Abbreviations: ALT, alanine aminotransferase; ANIT, α-naphthylisothiocyanate; AP, alkaline phosphatase; BF, bile flow; ETH, ethionine; EV, estradiol valerate; HTMP, 4-hydroxy-2,2,6,6-tetra-methylpiperidine-1-oxyl; LDH, lactate dehydrogenase; LT, leukotriene; LTB_4, LTC_4, LTD_4, LTE_4, leukotriene B_4, C_4, D_4 and E_4; LTE_4NAc, *N*-acetyl-leukotriene E_4; TCh, taurocholate.

development of cholestasis and hepatic leukotriene elimination, we measured the biliary secretion and metabolism of LTC_4 in isolated perfused rat livers under three different conditions of altered bile secretion. Paracellular permeability of the bile tract was increased by pretreating rats with ANIT [32], ETH* and EV [31]. The results indicate that changes in biliary secretion and metabolism of LTC_4 are early alterations in the development of cholestatic disorders and are caused by different pathogenetic mechanisms.

MATERIALS AND METHODS

Chemicals. [^{32}P]Orthophosphate and [^{14}C]sucrose were purchased from Du Pont GmbH (Bad Homburg, Germany), [^{14}C]taurocholate, [^{3}H]LTC_4, LTD_4, LTE_4 and LTE_4NAc from Amersham Buchler GmbH (Braunschweig, Germany). Unlabelled LTC_4 was a generous gift of Allergopharma Joachim Ganzer KG (Reinbek, Germany). ANIT was obtained from Eastman Kodak (Rochester, NY, U.S.A.), EV from Schering A.G. (Berlin, Germany), ETH from Aldrich (Milwaukee, WI, U.S.A.), HTMP from Sigma GmbH (Heidelberg, Germany), and taurocholic acid from Fluka AG (Buchs, Switzerland). All other substances and general reagents were from Merck A.G. (Darmstadt, Germany).

Treatment of animals. Male Wistar rats (Ivanovas, Kisslegg, Germany) were maintained at 12 hr light–dark cycles. Control rats and the animals treated with ANIT and EV received a standard laboratory diet (Altromin, Lage, Germany). Animals to be treated with ETH were fed a diet of low methionine 3 weeks before beginning the treatment. ANIT was dissolved in olive oil (200 mg/mL) and administered by gavage as a single dose of 250 mg/kg to rats weighing 250–300 g. Livers were isolated and perfused 6–7 hr later. EV dissolved in olive oil (1 mg/mL) was injected subcutaneously once a week for 3 months as described earlier [31]. These rats increased in weight only moderately, from 220–260 g to 240–280 g, during the treatment. ETH (100 mg/kg/day) was given for 4–6 weeks: half was dissolved in the drinking water, half was administered by gavage as a suspension in water three times a week. The dose in the drinking water was controlled by the volume of drinking. These animals remained at a body weight of 220–250 g during the treatment. Controls were given drinking water by gavage 3 days a week for 4–6 weeks to control for ETH treatment. They had a weight of 250–300 g at the end of treatment. The experiments were approved by an animal welfare comittee of the University of Tübingen.

Measurement of hepatocellular enzyme release. The activities of indicator enzymes of hepatic injury were measured by conventional pyridine nucleotide-linked optical tests. ALT (EC 2.6.1.2.) and AP (EC 3.1.3.1.) were determined in serum collected from the vena cava during the liver isolation procedure. LDH (EC 1.1.1.27.) in the perfusate of isolated liver was determined in samples taken 65 min after cannulation of the portal vein.

Isolated liver perfusion and measurement of determinants of bile secretion. Livers were isolated and perfused hemoglobin-free as described earlier [30, 34]. Krebs–Henseleit bicarbonate buffer, saturated with carbogen (95% O_2, 5% CO_2), was pumped into the portal vein at a constant rate of $\geq$3 mL/min/g liver. Bile was collected from polyethylene tubing (internal diameter 0.4 mm). Bile flow was determined by measuring the weight per time period of collection. Twenty five minutes after cannulation of the portal vein, ^{3}H-labelled LTC_4 was infused before the portal cannula at a rate of 70 pmol/min. From min 40 to min 65, perfusion medium containing 1 mM ^{14}C-labelled sucrose was recirculated. [^{14}C]-Taurocholate (290 nmol/min or 2000 nmol/min) was infused beginning at min 75. Basal bile flow was measured 30–40 min after cannulation of the portal vein. Oxygen concentration in the effluent was monitored by a Clark-type electrode calibrated by saturation of the perfusion medium with either nitrogen or carbogen.

Determination of LTC_4 metabolites in bile. From min 30 to 40 and from min 50 to 60, bile was collected for determination of [^{3}H]LTC_4 metabolites by HPLC analysis. To this end, fresh bile was immediately made up to 80% methanol by addition of methanol/water (9/1, pH 7.4, 1 mM HTMP, 0.5 mM EDTA), cooled on ice, and made oxygen-free by gassing with argon [35]. The mixture was adjusted to pH 7.4 with NH_3 and acetic acid and stored at $-20°$ for at least 3 hr. After centrifugation at 8000 *g* for 10 min the supernatant was dried under a nitrogen stream and redissolved in 30% methanol containing 1 mM HTMP and 1 mM EDTA, pH 5.6 to inject into the HPLC valve.

Reversed-phase HPLC (Beckman 110B) was performed using a C-18 spherisorb column (Beckman Ultrasphere 5 μm) and 65% methanol/0.1% acetic acid/1 mM EDTA (pH 5.6) as isocratic eluent at a flow rate of 1 mL/min. ^{3}H-Radioactivity was determined in 1 mL fractions, and retention times were compared with standard mixtures (LTC_4, LTD_4, LTE_4, and LTE_4NAc) run before and after bile analysis. Great care was taken to ensure that standards mixed with bile and treated like the radioactive probes had the same retention times.

Determination of glutathione in liver tissue and bile. Liver was freeze-clamped 130 min after start of perfusion, and a 20% homogenate was prepared for determination of glutathione (GSH). Bile was collected between 10 and 15 min after start of perfusion. Total glutathione (total of oxidized and reduced form) was assessed by the enzymic method of Tietze [36] using photometric determination of 2-nitro-5-thiobenzoic acid.

Treatment of data. Results are expressed as mean values ± standard deviation (SD). Statistical significance was estimated by use of the *t*-test following analysis of variance, $P \leq 0.05$ being considered significant. Linear regression was assessed by the least squares method.

RESULTS

Assessment of liver viability by hepatocellular enzyme release

In order to confirm the early, functional nature of

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Table 1. BF (μL/min/g liver) and stimulation of BF by TCh in isolated livers after different treatment

	Control (N = 9)	EV (N = 9)	ANIT (N = 9)	ETH (N = 7)
BF min 30–40	0.93 ± 0.15	0.98 ± 0.14	0.74 ± 0.28	0.63 ± 0.19*
BF min 70–75	0.86 ± 0.17	0.98 ± 0.23	0.57 ± 0.18*	0.39 ± 0.14†
	(N = 6)	(N = 6)	(N = 8)	(N = 7)
BF(TCh)min 80–85	1.23 ± 0.32	1.50 ± 0.30	0.82 ± 0.27	0.73 ± 0.34

Livers were isolated and perfused as described in Materials and Methods. TCh was infused at a rate of 290 nmol/min, 75 min after start of the perfusion. Maximal bile flow was recorded between 5 and 10 min after start of the bile acid infusion.
*$P < 0.05$ (df 5); †$P < 0.01$ (df 4) compared to controls.

Table 2. Distribution of [^{14}C]sucrose between bile and perfusate

	Control (N = 9)	EV (N = 8)	ANIT (N = 8)	ETH (N = 7)
Sucrose clearance (μL/min/g liver)	0.16 ± 0.04	0.34 ± 0.10‡	0.24 ± 0.07	0.19 ± 0.06
Sucrose bile/perfusate ratio	0.18 ± 0.07	0.36 ± 0.06	0.46 ± 0.26*	0.42 ± 0.11†

Sucrose clearance and bile/perfusate ratio were determined during recirculation 25 min after closing the perfusion system as described in Materials and Methods. Sucrose bile/perfusate ratio is the ratio of concentration of [^{14}C]sucrose in bile and perfusate.
*$P < 0.05$ (df 5); †$P < 0.05$ (df 4); ‡$P < 0.005$ (df 5) compared to controls.

the alterations of bile secretion, hepatocellular enzyme release was assessed. LDH was determined in the perfusate of isolated livers at the end of the recirculation period 65 min after cannulation of the portal vein. ALT and AP were measured in serum taken from rats during the liver isolation procedure. No increase was observed in enzyme concentrations in treated rats, which was similar to the results obtained in developing cholestasis induced by carmustine [37]. This indicates sustained viability of liver cells after the different treatments.

Characterization of changes in bile secretion.

Changes in bile secretion induced by ANIT, EV and ETH were characterized by bile flow and biliary permeability in isolated perfused livers of treated rats. In EV-treated livers, unstimulated bile flow determined between 30 and 40 min after cannulation of the portal vein was not lower than in controls (Table 1). In contrast, pretreatment with ANIT and ETH resulted in significant inhibition of bile flow. When TCh was infused at a rate of 290 nmol/min, bile flow was stimulated. Stimulation appeared minor after ANIT treatment whereas a considerable recovery of bile flow was observed in ETH-treated livers, although the differences did not reach a significant level.

Biliary access of sucrose was facilitated in all models (Table 2). Sucrose clearance was enhanced after EV. After ANIT and ETH, the increase in sucrose clearance was not significant despite an elevated bile/perfusate ratio since bile flow was decreased. This increase in sucrose access into bile is in accord with earlier reports on ANIT cholestasis [30, 32] on the effect of estradiolvalerate [31], and with results on ETH cholestasis*. Since it has also

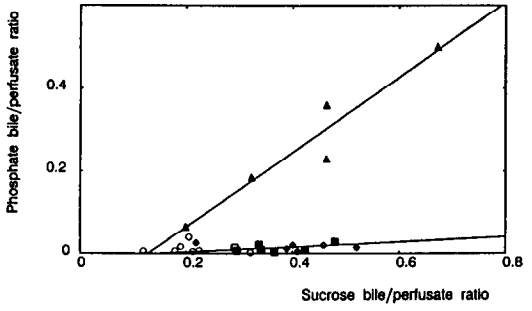

Fig. 1. Correlation between bile/perfusate ratio of [^{14}C]-sucrose and the ratio of inorganic [^{32}P]phosphate in isolated perfused livers. The bile/perfusate ratios are given as the equilibrium concentrations of the markers reached after 25 min of recirculation. Pretreatment of rats is indicated by filled triangles (ANIT), filled squares (EV), filled rhombs (ETH), and open circles (controls). A correlation coefficient of $R = 0.96$ was calculated for the regression line for the ANIT pretreatment.

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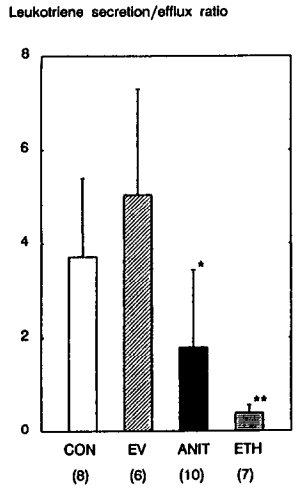

Fig. 2. Ratio between biliary secretion and efflux into perfusate of [^{3}H]leukotriene C_4-derived radioactivity in isolated rat liver. Rats were pretreated with EV, ANIT, or ETH and compared to controls, as described in Materials and Methods. ^{3}H-Labelled LTC_4 was infused into the portal vein at a final concentration of 2.5 nM and the distribution of ^{3}H-radioactivity between perfusate and bile was monitored 45–50 min after beginning the infusion. Numbers in parentheses pertain to the numbers of experiments. * $P \leq 0.05$ (df 5); ** $P \leq 0.02$ (df 4).

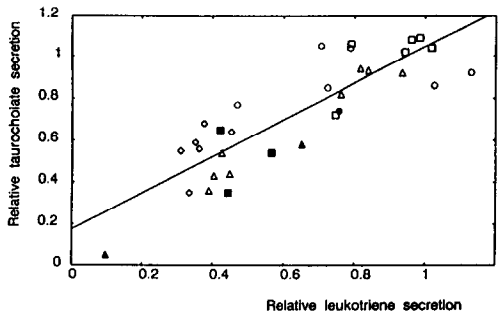

Fig. 3. Correlation between [^{14}C]TCh and [^{3}H]LT secretion rates into bile in isolated perfused livers. Fractional secretion rates were determined between 10 and 15 min after commencing the TCh infusion into isolated livers of control rats (circles) and rats pretreated with ANIT (triangles), EV (squares), and ETH (rhombs). The infusion rate of TCh was approx. 50 (open symbols) or 200 nmol/min/g liver (filled symbols). The correlation coefficient of the regression line is $R = 0.86$.

been suggested that inorganic phosphate may be a marker of permeability of the bile tract [32, 38], [^{32}P]phosphate in bile and perfusate was determined as well. Only after ANIT treatment was there a significant increase in ^{32}P access into bile. As demonstrated in Fig. 1, the bile/perfusate ratios of [^{32}P]phosphate and [^{14}C]sucrose were correlated after ANIT and EV pretreatment, there being a much higher phosphate ratio after ANIT.

Changes in [^{3}H]LTC_4 and [^{14}C]TCh distribution

The capacity to secrete [^{3}H]LTC_4 was impaired after both ANIT and ETH treatment. Instead, a higher fraction of infused radioactivity appeared in the perfusate after passing the liver and the ratio between [^{3}H]LTC_4-derived radioactivity in bile and in the effluent was decreased (Fig. 2). In contrast, after EV no decrease in this ratio was observed.

Bile flow and secretion of LTs were interrelated. Below a bile flow of approx. 1 μL/min/g liver there was a linear correlation between bile fluid formation and biliary secretion of [^{3}H]LTC_4-derived radioactivity ($R = 0.85$, data not shown). Accordingly, the fraction released into the perfusate increased when bile flow was diminished.

The relative secretion rate of LTC_4 was correlated with that of TCh (Fig. 3). TCh was infused at a rate of 290 nmol/min in most experiments. In some, however, TCh was infused at a much higher rate (2000 nmol/min). Under these conditions the relative secretion rates of both cholephilic compounds also fitted well into the correlation. Since the absolute secretion rate of TCh was much higher in these cases, this demonstrates that infusion of 290 nmol/min was well below the maximum secretion rate for TCh. Thus, changes in TCh distribution are caused by other factors than an alteration of maximum secretion capacity.

Role of biliary permeability for LT distribution

Given the known effects of ANIT [32] and EV [31] on biliary permeability and the increased bile/perfusate ratio of sucrose after ETH treatment, we examined whether a bilio-sinusoidal reflux might contribute to the altered distribution of [^{3}H]LTC_4-derived radioactivity. [^{14}C]Sucrose clearance was used as an estimate for biliary permeability. As shown in Fig. 4, a very different behaviour was observed after the various pretreatments. After ANIT, both the biliary secretion rate (a) and the venous efflux rate (b) of ^{3}H-radioactivity were correlated with sucrose clearance (a regression line is shown only for this group). A similar correlation was observed in the ANIT model between ^{3}H-distribution and [^{32}P]phosphate clearance (results not shown). In contrast, no correlation between sucrose access into bile and LT secretion was found after treatment of rats with EV or ETH.

Metabolites of LTC_4 in bile

The metabolites of [^{3}H]LTC_4 in bile were determined by HPLC fractionation. Results from the bile samples collected during the early phase of LTC_4 infusion, i.e. under non-recirculating conditions, are shown in Table 3. Similar results were obtained when bile was collected after 20 min of recirculation of the medium (not shown) except that the relative amount of polar metabolites was slightly higher. In control livers, 36.5% of biliary ^{3}H-radioactivity consisted of LTC_4, approximately the same amount of polar metabolites. Only 11.3% were LTD_4. After all pretreatments the fraction of LTD_4 was significantly higher than in controls, at the expense of LTC_4 and polar metabolites. This effect was most prominent after EV pretreatment

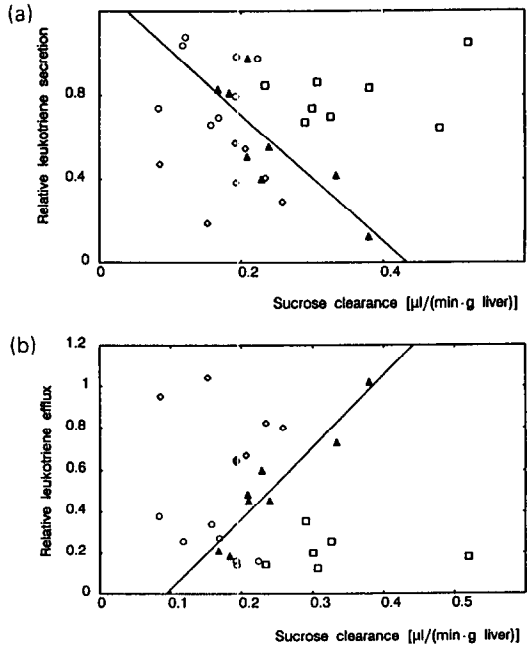

Fig. 4. Relationship between [^{14}C]sucrose clearance and biliary secretion (a) and venous efflux (b) of [^{3}H]LT C_4-derived radioactivity in isolated perfused livers. Sucrose clearance is given as the equilibrium value of the marker reached after 25 min of recirculation and ^{3}H output as that after 45 min of [^{3}H]LT C_4 infusion. Pretreatment of rats is indicated by filled triangles (ANIT), open squares (EV), open rhombs (ETH), and open circles (controls). The regression line was calculated only for the ANIT-pretreated livers. Correlation coefficients for this group are $R = 0.81$ (a) and $R = 0.94$ (b).

when both LTC_4 and polar metabolites were significantly diminished. LTE_4 and LTE_4NAc were low in bile of isolated livers and were not significantly altered by the different treatments.

Glutathione content of liver and bile

The concentration of glutathione in bile, determined in the first sample collected during liver perfusion, was significantly diminished after ETH and EV treatment (Table 4). In contrast, no difference was observed after ANIT. To determine the reason for the lowering of biliary glutathione secretion, glutathione in livers was analysed at the end of the perfusions. Only after ETH treatment can the decrease in biliary glutathione be caused by a decrease in cellular glutathione, whereas after EV pretreatment total glutathione in livers was even higher than in controls. The fraction of LTD_4 of biliary [^{3}H]LTC_4-derived radioactivity was negatively correlated with biliary glutathione concentration ($R = 0.83$, data not shown).

DISCUSSION

Alterations of bile secretion and cysteinyl LT distribution by cholestatic compounds

Cysteinyl LTs are rapidly taken up into hepatocytes as demonstrated in isolated cells [20]. Uptake and concentrative transport into bile were demonstrated in rats [24, 25, 39] and in isolated perfused rat livers [7, 40–43]. In this report, we have studied the uptake, metabolism and secretion of [^{3}H]LT in isolated perfused livers after pretreatment with agents inducing increased permeability of the bile tract. ANIT [29, 30, 32] and long-term EV treatment [29, 31] were previously shown to increase the permeability of the bile tract, resulting in possible bilio-sinusoidal reflux of cholephilic compounds. ETH treatment also facilitates the access of sucrose into bile, a result confirmed in this study by an elevated bile/perfusate ratio of sucrose.

After pretreatment with ANIT and ETH, the distribution of LTC_4 in isolated livers was altered. In contrast, no decrease in total LT secretion into bile was observed after EV pretreatment despite considerably increased biliary sucrose clearance. Thus, increased permeability to sucrose is not sufficient to inhibit overall transport of cysteinyl LTs between sinusoidal and biliary spaces in the whole organ. On the other hand, bile flow was reduced by both ETH and ANIT and distribution of LTC_4 and its metabolites was correlated with bile flow.

Paracellular reflux and charge-selectivity of permeability changes

Inorganic phosphate in bile was earlier proposed as a marker of paracellular permeability in ANIT

Table 3. Relative portion of [^{3}H]LT metabolites in bile (in [%] of total radioactivity)

	Control (N = 7)	EV (N = 6)	ANIT (N = 9)	ETH (N = 5)
LTC_4	36.6 ± 5.4	16.7 ± 10.8*	34.7 ± 9.8	19.3 ± 7.6
LTD_4	11.3 ± 1.8	45.4 ± 11.9§	20.1 ± 7.7†	32.5 ± 4.4‡
LTE_4	1.02 ± 0.60	0.73 ± 0.84	1.65 ± 1.39	2.66 ± 1.92
LTE_4NAc	4.17 ± 1.34	5.71 ± 1.01	5.05 ± 1.76	4.06 ± 1.44
Polar metabol.	37.2 ± 5.6	25.2 ± 4.4*	32.1 ± 6.3	36.5 ± 5.7

Bile was collected between 5 and 15 min after start of the infusion of [^{3}H]LTC_4 into isolated livers, and analysed for cysteinyl leukotrienes by HPLC separation as described in Materials and Methods.

*$P < 0.05$ (df 3); †$P < 0.05$ (df 4); ‡$P < 0.05$ (df 2); §$P < 0.01$ (df 3) compared to controls.

Table 4. Glutathione (GSH) content (total of oxidized and reduced form) in bile and liver tissue after isolated perfusion

	Control	EV	ANIT	ETH
Total GSH in bile (μmol/L)	3438 ± 799 (N = 6)	484 ± 513‡ (N = 7)	3236 ± 557 (N = 4)	308 ± 299* (N = 6)
Total GSH in liver (nmol/g liver)	4232 ± 1154 (N = 7)	5295 ± 553 (N = 7)	3081 ± 479 (N = 5)	2405 ± 714† (N = 9)

Livers were freeze-clamped 130 min after start of perfusions. Bile was collected 10–15 min after start of perfusions. GSH was assessed as described in Materials and Methods.
*$P < 0.02$ (df 2); †$P < 0.01$ (df 4); ‡$P < 0.005$ (df 3).

cholestasis [32, 38]. In this study, we found a markedly increased access of phosphate into bile only after ANIT pretreatment, but not in livers pretreated with ETH or EV. These differences may be accounted for by different degrees of charge-selectivity of tight junctions [44] being preserved. The negatively charged phosphate ion can enter bile only if the charge-selectivity of the paracellular pathway is reduced. Accordingly, a correlation between clearance of both sucrose and inorganic phosphate and venous efflux of LTC_4-derived radioactivity was only observed after ANIT treatment, in line with a paracellular reflux of the negatively charged cysteinyl-LTs. This cannot be expected in the more charge-selective permeability changes following EV and ETH pretreatment, when the permeability to the negatively charged phosphate was only slightly increased or unchanged.

Impairment of transcellular transport

In contrast, intracellular events or inhibition of transport carriers should account for the impairment of biliary excretion of cysteinyl LTs and taurocholate in cholestasis by ethionine. Transport of cysteinyl LTs across the canalicular membrane was shown to be impaired in mutant rats, with a defective anion carrier in which the biliary transport of bilirubin and sulfobromophthalein is inhibited as well [45]. Transport of cysteinyl LTs via this carrier was shown to be energy-dependent [19]. Under our conditions, the impairment of biliary elimination in the intact organ resulted in increased concentration in the sinusoidal compartment.

LTC_4 metabolism and biliary glutathione

Pretreatment of rats also resulted in a changed pattern of LTC_4 metabolites in bile. The most striking feature was the increased formation of LTD_4. Two mechanisms might account for this change: either the biliary γ-glutamyltransferase reaction was influenced or the time of contact of the enzyme with LTC_4 was prolonged. This could be a result of lowered bile flow. However, in the case of estradiolvale rate, bile flow was not lower than in controls in this study. Increased formation of LTD_4 was also found under hypoxic perfusion of isolated livers [35]. However, hypoxia due to inhibition of oxygen uptake was not observed in the conditions of cholestasis in our study. Interestingly enough, the increase in LTD_4 formation was closely correlated with a decrease in biliary glutathione. These findings are consistent with the report of Wettstein *et al.* [43], suggesting equilibrium distribution of the glutamyl residue between glutathione and LTC_4 due to biliary γ-glutamyltransferase. Accordingly, this relationship was seen only in bile and not between intracellular glutathione and LT metabolites.

LT retention and liver injury

It can be predicted that decreased elimination via bile increases the LT content of the liver, as shown in cholestasis induced by phalloidin, bile duct ligation, and endotoxin [21]. Our results demonstrate altered bilio-sinusoidal distribution of LTs in early stages of liver injury. Increased formation of LTD_4 may contribute to enhanced toxicity, as it has been shown that this metabolite is a mediator of galactosamine/endotoxin-induced hepatitis [46]. Liver content of LTs was not measured in the present experiments. However, altered distribution would be expected to result in higher sinusoidal and venous equilibrium concentration *in vivo* as well.

LT kinetics and subclinical cholestasis

In conclusion, the present results demonstrate marked changes in biliary elimination of cysteinyl LTs under different conditions of altered bile secretion. These conditions are characterized by differential changes in bile flow and in the permeability of the bile tract, whereas commonly used enzymic markers of cholestasis and liver injury in humans were not increased. These conditions can therefore be qualified as "subclinical" cholestasis. Only after ANIT pretreatment could the increase in paracellular permeability account for the change in distribution, although it might affect metabolism of cysteinyl LTs; on the other hand, events within parenchymal or ductular epithelial cells appear to be responsible for the changes after ETH and EV. It is proposed that different charge selectivity of the increased permeability, as indicated by inorganic phosphate access into bile, accounts for these differences.

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