Novel 3-Hydroxylated Leukotriene B$_4$ Metabolites from Ethanol-Treated Rat Hepatocytes

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Coincubations of radiolabeled leukotriene B$_4$ (LTB$_4$) and ethanol with isolated rat hepatocytes led to formation of one dihydroxylated and two novel β-oxidized metabolites of LTB$_4$. The major radioactive peaks from reverse-phase–high performance liquid chromatography (RP–HPLC) eluted with material absorbing UV light maximally at 270 nm, with shoulders at 260 and 280 nm, indicating retention of the conjugated triene structure of the parent molecule in each metabolite structure. Following purification, catalytic reduction, and derivatization, mass spectrometric analysis revealed that all three metabolites were hydroxylated at the C-3 carbon atom based on characteristic ions at m/z 201 and 175 in the electron ionization mass spectra of the metabolites. Negative-ion electron capture mass spectrometry of the metabolites as pentafluorobenzyl (PFB) ester, trimethylsilyl ether derivatives aided structural characterizations while revealing interesting fragmentations. A ketene-containing ion appeared to result from the loss of both PFB groups (one as PFB alcohol), while a lactone alkoxide ion appeared to result following loss of PFB and bis(trimethylsilyl) ether. From these data three novel LTB$_4$ metabolites were suggested to be 3,20-dihydroxy-LTB$_4$ (3,20-diOH-LTB$_4$), 3-hydroxy-18-carboxy-LTB$_4$ (3-OH-18-COOH-LTB$_4$), and 3-hydroxy-16-carboxy-LTB$_3$ (3-OH-16-COOH-LTB$_3$). The significance of the almost exclusive formation of these 3-hydroxylated LTB$_4$ metabolites in the presence of ethanol is currently unknown, but may result from interrupted β-oxidation from the C-1 carboxyl moiety. (*J Am Soc Mass Spectrom 1992, 3, 762–768*)

L eukotriene B$_4$ (LTB$_4$, 5(S),12(R)-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic acid) is a lipid mediator enzymatically derived from arachidonic acid [1, 2]. Several investigators have demonstrated numerous biological activities of this endogenous substance which supports its putative role in inflammation and several pathophysiological conditions such as arthritis and psoriasis [3]. This potent substance is extensively metabolized in vivo [4] and several metabolic studies have utilized isolated cells or tissues as model systems for studying LTB$_4$ metabolism [5–9]. LTB$_4$ is ω-oxidized in both neutrophils and hepatocytes to 20-OH-LTB$_4$ (5,12,20-trihydroxy-6,8,10,14-eicosatetraenoic acid) and 20-COOH-LTB$_4$ (5,12-dihydroxy-6,8,10,14-eicosatetraen-1,20-dioic acid) [5–9]. Unlike neutrophils, which utilize a specific cytochrome P-450 [10, 11] or aldehyde dehydrogenase [12], hepatocytes appear mainly to catalyze this oxidation with alcohol dehydrogenase and aldehyde dehydrogenase [13, 14]. Effects of ethanol (a favored substrate for alcohol dehydrogenase) on hepa-

tocyte metabolism of LTB$_4$ in other laboratories have raised questions about the possible effects of ethanol on the production of LTB$_4$ metabolites recently characterized in our laboratory [15].

Early studies of LTB$_4$ metabolism in isolated rat hepatocytes revealed ω-oxidation to 20-OH and 20-COOH-LTB$_4$ followed by β-oxidation to 18-COOH-LTB$_4$ (5,12-dihydroxy-6,8,10,14-octadecatetraen-1,18-dioic acid) [7]. Further β-oxidative chain-shortening and amino acid conjugation resulted in 16-COOH-LTB$_3$ (5,12-dihydroxy-6,8,10-hexadecatrien-1,16-dioic acid) and 18-tauro-LTB$_4$, respectively, as previously reported [15]. Interestingly, production of these β-oxidized metabolites was minimal when 44 mM ethanol was present in the incubation mixture. Instead, other LTB$_4$-related, radioactive, UV-absorbing chromatographic peaks appeared, representing different metabolites based on different high performance liquid chromatography (HPLC) retention times. In contrast to previous studies which showed substantial inhibition of hepatocyte LTB$_4$ metabolism by ethanol [14], this study revealed extensive metabolism to previously unidentified metabolites.

The purpose of this study was to structurally characterize ethanol-dependent LTB$_4$ metabolites by using

mass spectrometry. Analyses of both positive and negative ions were complementary for structure elucidation purposes, and several characteristic ions were identified from these hydroxylated carboxylic acids.

Experimental

Materials. LTB$_4$ was kindly provided by Dr. Robert Zipkin (Biomol Research Labs, Plymouth, PA) and (5,6,8,9,11,12,14,15-[^{3}H]$_8$)-LTB$_4$, 150–200 Ci/mmole was purchased from Dupont-New England Nuclear. Reverse-phase–HPLC (RP–HPLC) columns (150 × 4.6 mm Apex II ODS) were from Jones Chromatography (Columbus, OH). HPLC solvents were from Fisher Chemical Company (Fairlawn, NJ). Octadecysilyl (ODS) SepPak cartridges were from Waters Associates (Milford, MA). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Supelco (Bellefonte, PA). Diazald, used to prepare ethereal solutions of diazomethane, was obtained from Aldrich Chemical Co. (Milwaukee, WI).

Methods. Hepatocytes were isolated by the collagenase method of Berry and Friend [16] with slight modifications. Male and female Sprague-Dawley rats (200–500 g) were anesthetized with pentobarbital (65 mg/kg). After opening the abdomen to expose the portal vein, the liver was perfused with Ca^{2+}-free buffer (37°C, oxygenated) and removed from the animal. The liver was then perfused with 35 u/ml collagenase (Type II, Worthington, Freehold, NJ) and 1 mM Ca^{2+}-containing oxygenated buffer for 15 min. After a 5 min washout with collagenase/Ca^{2+}-free buffer, hepatocytes were freed from the liver and purified by four steps of differential centrifugation using a separate wash buffer each time. The hepatocytes were resuspended in an incubation buffer containing 129 mM NaCl, 5.2 mM KCl, 3 mM Na$_2$HPO$_4$, 0.9 mM MgSO$_4$, 1.1 mM CaCl$_2$, 4 mM glucose, 10 mM tris(hydroxymethyl)aminomethane, equilibrated with 95% O$_2$/5% CO$_2$, pH 7.4. Cell viabilities were > 85% as determined by trypan blue exclusion.

Hepatocyte incubations were carried out with 12 μM LTB$_4$, 3.5 μCi [^{3}H$_8$]LTB$_4$, 1.25 × 10^8 hepatocytes/25 ml, 44 mM ethanol (0.2% w/v) for 30–60 min. The LTB$_4$ and ethanol were added simultaneously, premixed in incubation buffer. Flasks were filled with 95% O$_2$/5% CO$_2$ and then sealed for the duration of the incubations. Incubations were terminated by the addition of 4 vol of cold ethanol (−20°C). After 2 h at −70°C, the ethanolic suspension was centrifuged to pellet cell debris and precipitated proteins. The supernatant was dried under vacuum and the residue dissolved in water. Following acidification with acetic acid, an aliquot was injected onto an RP–HPLC column and monitored for UV absorbance and radioactivity in tandem (HP-1040A photodiode array detector, Hewlett-Packard, Palo Alto, CA; Flow One/Beta, Radiomatic, Tampa, FL). The remainder of

the material was then subjected to solid-phase extraction utilizing a 5-ml 0.1% acetic acid wash followed by methanol elution of lipophilic LTB$_4$ metabolites. This methanol fraction was dried under vacuum and separated by RP–HPLC. The first stage of RP–HPLC (system 1) utilized a linear gradient from 50% solvent A (16.6 mM ammonium formate adjusted to pH 4.5 with HCl) to 100% solvent B (methanol/solvent A/acetic acid, 90 : 10 : 0.1) in 30 min at a flow rate of 1 ml/min. LTB$_4$ metabolites were collected manually based on their UV absorbance and rechromatographed individually by using a linear gradient from 100% H$_2$O to 50% acetonitrile in 50 min at 1 ml/min (system 2). Metabolites were again collected manually and UV spectroscopy was performed on purified metabolites by using an HP-8452A UV spectrophotometer (Hewlett Packard).

Metabolites derivatized for electron ionization (EI) (70 eV) were methylated with ethereal diazomethane for 5 min in methanol, dried under a stream of nitrogen, and purified as methyl esters by using HPLC system 2. The purified methyl esters were then reduced by catalytic hydrogenation with platinum oxide and hydrogen gas for 90 s at −20°C. The reduced methyl esters were then reacted with 100 μl of 50% BSTFA in acetonitrile for 20 min at 60°C. Electron ionization gas chromatography-mass spectrometry (GC-MS) was carried out on a Finnigan SSQ-70B mass spectrometer (Finnigan Mat, San Jose, CA) interfaced with a DB-1 capillary column (10 m × 0.25 mm inside diameter, 0.2 μm film thickness programmed from 100 to 320°C at 15°C/min using helium as carrier gas. Metabolites derivatized for negative ion electron capture ionization (ECI) mass spectrometry were dissolved in 30 μl methanol to which 70 μl acetone was added, followed by 20 μl pentafluorobenzylbromide (Aldrich) and 10 μl N,N-diisopropylethylamine (Aldrich). The mixtures were heated to 60°C for 15 min, dried under vacuum, dissolved in 50 μl MeOH, and the derivatives were purified as their UV-absorbing, pentafluorobenzyl esters by using HPLC system 2. The purified benzyl esters were then treated with BSTFA as described above for the methyl ester, trimethylsilyl ether derivatives. Negative ion GC-MS analyses were performed on a Finnigan SSQ-70B using methane as moderating gas at 0.3 torr source pressure. GC conditions were identical to those described above.

Results

After LTB$_4$ was incubated with rat hepatocytes for periods up to 1 h in the presence of 44 mM ethanol, no LTB$_4$ or 3-OH-LTB$_4$ remained, in contrast to the result when shorter incubation times were employed, as previously reported [17]. The coelution of radioactive and UV-absorbing LTB$_4$ metabolites is illustrated in Figure 1. Three major peaks, designated H$_E$, H$_I$, and H$_L$, are indicated along with their UV absorbance spectra in the insets to this figure. The retention time of 3-OH-

LTB$_4$ is indicated by the arrow at 28 min. A separate experiment with a shorter incubation time (15 min) resulted in a major metabolite at this retention time (data not shown). LTB$_4$ metabolites which normally appear in the absence of ethanol were not apparent by comparison of HPLC retention times. Each labeled metabolite represents a novel substance generated when ethanol was present in the incubation; together the new metabolites correspond to 24% of the initial LTB$_4$ added to the hepatocytes.

The structure of 3-OH-LTB$_4$ was determined by GC-MS analysis following reduction and derivatization. The EI mass spectrum revealed ions which correlated with those seen in a previously published mass spectrum [7] at m/z 477, 395, 387, 311, 247, 215, 201 (base peak), 175, and 147. The ECI mass spectrum of derivatized 3-OH-LTB$_4$ contained a base peak at m/z 567 (M-181, loss of PFB) and ions at m/z 477, 387, and 297, suggesting losses of up to three TMSOH groups from the M-181 ion of this derivative which had an equivalent chain length (ECL) of 23.9. The presence of three hydroxyl groups on the underivatized metabolite, molecular weight information indicating four intact double bonds, the UV absorbance spectrum (data not shown), and a similar EI mass spectrum and gas chromatographic equivalent chain length (ECL) (25.3 versus 25.5) of the reduced metabolite identified previously (7), are all consistent with identification as 3,5,12-trihydroxyeicosatetraenoic acid (3-OH-LTB$_4$). The stereochemistry of the 3-hydroxy group was not determined.

Metabolite H$_E$. The ECI mass spectrum (Figure 2a) of the PFB, trimethylsilyl (TMS) derivative of metabolite H$_E$ (ECL = 26.5) revealed an M-181 ion at m/z 655 (base peak). Because this ion appeared 88 u higher
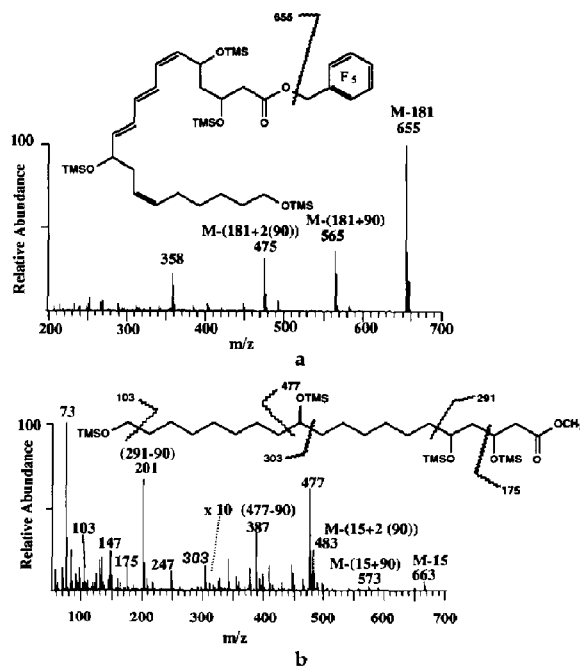

Figure 2. Mass spectra of metabolite H$_E$ under negative- and positive-ion conditions. (a) ECI-MS analysis of H$_E$ as the PFB, TMS derivative; (b) EI-MS analysis of H$_E$ as the reduced, methylated, TMS derivative.

than the M-181 ion of derivatized 3-OH-LTB$_4$, this suggested that H$_E$ contained an additional hydroxyl group in the underivatized molecule. Other major ions appeared at m/z 565 (M-181-90, loss of PFB and TMS-OH) and m/z 475 (M-181-90-90, loss of PFB and 2 TMSOH groups).

Electron ionization mass spectrometric analysis of metabolite H$_E$ as the methyl ester, TMS derivative following catalytic hydrogenation (Figure 2b) revealed ions at m/z 663 (M-15, loss of CH$_3$) and other significant ions at m/z 483 (M-15-90-90, loss of CH$_3$, and two TMSOH groups), m/z 477 (fragmentation indicated), and m/z 387 (477-90), m/z 303 (fragmentation indicated), and m/z 201 (291-90, cleavage as indicated followed by loss of TMSOH). The ECL of this derivative of metabolite H$_E$ was determined to be 28.2. Based on the UV absorbance spectrum, the presence of an additional hydroxyl group in the underivatized metabolite, and the ions appearing in the EI mass spectrum corresponding to ions seen previously in the EI mass spectrum of reduced, derivatized 3-OH-LTB$_4$ (m/z 477, 201, and 175), metabolite H$_E$ was assigned the structure 3,5,12,20-tetrahydroxyeicosatetraenoic acid (3,20-dihydroxy-LTB$_4$).
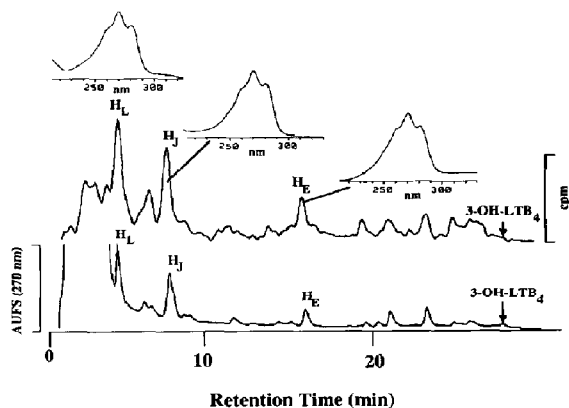

Figure 1. HPLC chromatograms of LTB$_4$ metabolites illustrating coeluting UV-absorbing and radioactive peaks obtained by tandem UV-absorption/scintillation counting. The UV-absorption spectra of metabolites H$_L$, H$_J$, and H$_E$ are shown in the insets. The retention time for the 3-OH-LTB$_4$ metabolite is indicated.

Metabolite H$_J$. When metabolite H$_J$ was derivatized and analyzed by GC-MS under negative ion conditions

(Figure 3a), ions appeared at m/z 749 (M-181), m/z 659 (M-181-90), m/z 587 (M-181-162, loss of TMS-O-TMS), and m/z 551 (M-181-198, loss of PFB and PFB-OH). The M-181 ion and the presence of M-181-162 and M-181-198 fragment ions suggested that H_J might be a dicarboxylic acid metabolite of LTB_4. Similar mass spectral behavior has been observed for 20-COOH-LTB_4 and 18-COOH-LTB_4 derivatives [15]. The M-181 ion was 88 u higher than the corresponding M-181 ion appearing in the ECl mass spectrum of derivatized 18-COOH-LTB_4 (data not shown), suggesting the presence of an additional hydroxyl group in the underivatized molecule. The PFB, TMS derivative of metabolite H_J had an ECL of 29.6

Analysis of reduced, derivatized metabolite H_J by EI GC-MS (Figure 3b) revealed several ions identical to those seen in the analogous analysis of metabolites H_E and 3-OH-LTB_4 (m/z 477, 201, and 175), suggesting an identical structure from the C-12 to C-1 portion of this metabolite, in particular the presence of a hydroxyl moiety at the C-3 carbon. Because the ion at m/z 231 was seen during EI mass spectrum of reduced 18-COOH-LTB_4 [7], this suggested that the structure of the C-12 to C-18 portion of metabolite H_J was identical to that of 18-COOH-LTB_4. Reduced H_J-Me-TMS had an ECL of 26.3. From these observations, the M-15 (loss of CH_3) ion at m/z 591, the negative ion mass spectrum, and the UV absorbance spectrum, it was con-

cluded that metabolite H_J was 3,5,12-trihydroxy-octadecatetraene-1,18-dioic acid (3-OH-18-COOH-LTB_4).

Metabolite H_L. Analysis of the PFB, TMS derivative of metabolite H_L (ECL = 27.9) by ECl GC-MS (Figure 4a) revealed four major ions (m/z 723, 633, 561, and 525), each of which was 26 u less than the corresponding ion seen in the ECl spectrum of derivatized metabolite H_J above. Based on the previous study [15] which showed an analogous difference in masses (26 u) between the mass spectra of 18-COOH-LTB_4-PFB-TMS and 16-COOH-LTB_3-PFB-TMS, H_L was hypothesized to be a β-oxidized chain-shortened, reduced metabolite of H_J.

Electron ionization GC-MS analysis of the reduced methylated, trimethylsilylated derivative of H_L (Figure 4b, ECL = 24.6) revealed ions at m/z 547 (M-31), m/z 477, and m/z 389 (fragmentations indicated), m/z 387 (477-90), m/z 383 (M-15-90-90), m/z 299 (389-90), m/z 203, and m/z 201 (291-90). Again, the presence of an m/z 201 ion suggested an hydroxyl moiety on the C-3 carbon of the underivatized metabolite. The ion at m/z 203 was analogous to the m/z 203
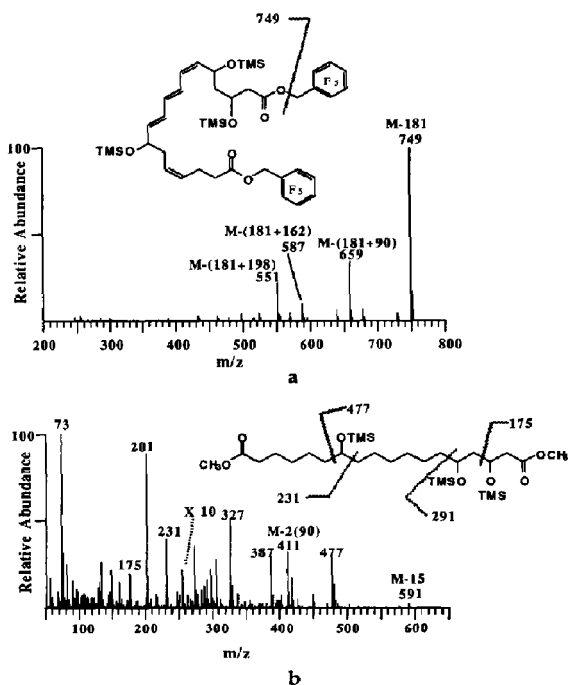

Figure 3. GC-MS analysis of metabolite H_J under negative- and positive-ion conditions. (a) ECl mass spectrum of metabolite H_J as the PFB, TMS derivative; (b) EI mass spectrum of reduced H_J as the methyl ester, TMS ether derivative.
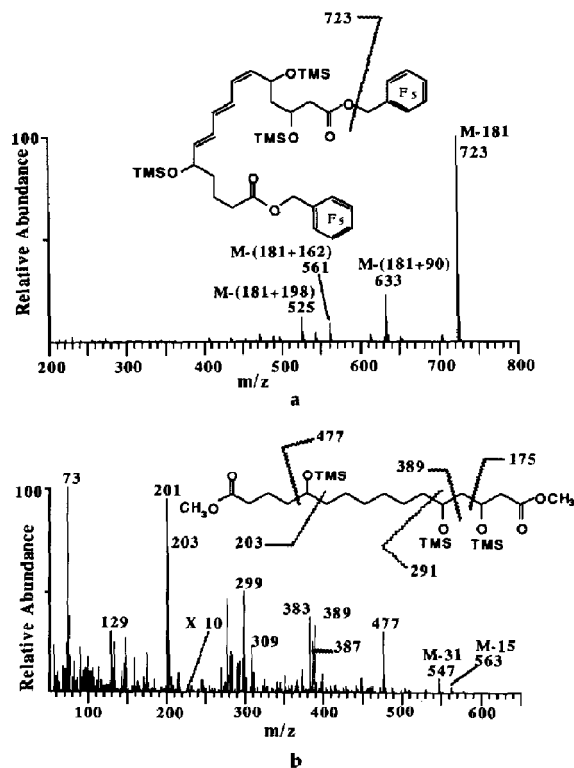

Figure 4. GC-MS analysis of metabolite H_L under negative- and positive-ion conditions. (a) ECl mass spectrum of metabolite H_L as the PFB, TMS derivative; (b) EI mass spectrum of reduced H_L as the methyl ester, TMS ether derivative.

ion observed during EI analysis of reduced LTB$_4$-Me-TMS, except that with the H$_L$ derivative, this ion originates from the opposite end of the molecule. The UV absorbance spectrum and both negative and positive ion mass spectra were consistent with identification of metabolite H$_L$ as 3,5,12-trihydroxyhexadecatriene-1,16-dioic acid (3-OH-16-COOH-LTB$_3$).

Certain fragment ions appearing in both negative and positive ion mass spectra served critical roles in the structural characterization of these metabolites. In the ECl spectra of two metabolites, an [M-181-198] ion appeared, suggesting that these metabolites had common structural features. The [M-181-198]$^-$ ions could be accounted for if both metabolites were in fact dicarboxylic acids. A mechanism for the origin of these ions could involve ketene formation remote to the ionized carboxylate resulting from the loss of a PFB group (Scheme I). Another common ion appeared in the ECl mass spectra at [M-181-162]$^-$ for all metabolites including LTB$_4$ itself (data not shown). When [^{2}H$_{18}$]BSTFA was used for the derivatization, this ion was observed to lose two TMS moieties, consistent with formation of bis(trimethylsilyl) ether (TMS-O-TMS). We suggest that the driving force for this rearrangement is favorable attack of the carboxylate anion at C-5 with lactone formation and the anionic charge site moved to the oxygen at C-12 (Scheme II).

Two particular ions in the EI mass spectra of reduced, methylated, trimethylsilylated LTB$_4$ metabolites served as diagnostic indicators of a 3-hydroxy substituent present in the molecules. These ions, m/z 201 and 175, were evident in all of the EI mass spectra presented, as well as the EI spectrum of reduced 3-OH-LTB$_4$-Me-TMS published previously [7]. The m/z 201 ion is proposed to originate from a loss of trimethylsilylanol from the m/z 291 fragment ion, as illustrated in Scheme III. The formation of this latter ion in LTB$_4$ has been studied previously [18]. Though not as prevalent, the m/z 175 ion is likely an α-cleavage product.

Discussion

The relatively low concentrations of ethanol encountered in human beings during moderate ingestion of alcoholic beverages (10–50 mM) is known to exert profound central nervous system effects. Less well understood are the effects of these low concentrations of ethanol on biochemical events. Chronic ethanol in-
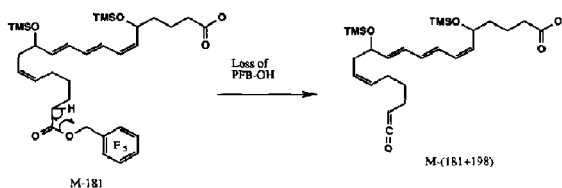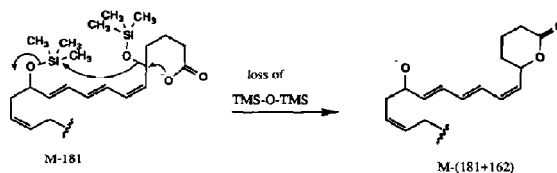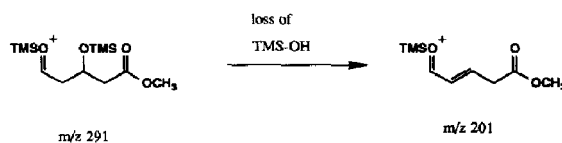

Scheme II

gestion by humans is known to lead to significant impairment of the immune defense system as well as tissue injury [19]. For example, chronic alcohol ingestion results in liver damage and alcoholic hepatitis in some cases. Alcoholic hepatitis is manifest by infiltration of polymorphonuclear leukocytes into the liver parenchyma with concomitant damage to hepatocytes following activation of the neutrophil [20]. Considering the fact that leukotriene B$_4$ is known to be a potent chemotactic agent which can recruit neurophils into a tissue, we have been interested in the effect of ethanol on leukotriene B$_4$ synthesis and metabolism.

Ethanol caused a qualitative alteration of the metabolite profile resulting from isolated rat hepatocyte preparations exposed to exogenous leukotriene B$_4$. The biochemical pathways are summarized in Figure 5. Extensive metabolism of LTB$_4$ in the presence of ethanol resulted in the production of three novel metabolites. Previously, LTB$_4$ was determined to undergo ω-oxidation and β-oxidation in hepatocytes, at least to 16-COOH-LTB$_3$ [7, 15]. Recent reports regarding the involvement of alcohol dehydrogenase and aldehyde dehydrogenase in hepatocyte conversion of 20-OH-LTB$_4$ to 20-COOH-LTB$_4$ [13, 14] led to studies [17] which were consistent with the findings of Keppler and co-workers [14] that ethanol cause a "crossover effect" on relative amounts of ω-oxidized LTB$_4$ metabolites produced in hepatocytes. In contrast to their studies, which included 0.4 mmol/l arachidonate and 5 g/l bovine serum albumin in the incubation buffer to slow metabolism, our studies displayed more extensive metabolism and the production of several LTB$_4$ metabolites.

Little is known about the nature of the 3-hydroxylation reaction and accumulation of such products in the presence of ethanol. It likely represents a product formed from the first two steps of one round of β-oxidation from the carboxy terminus of LTB$_4$. However, we cannot rule out the possibility that a 3-hydroxylation reaction might be mediated by another oxidase such as cytochrome P450. Considering a β-oxidation mechanism, further oxidation of the 3-
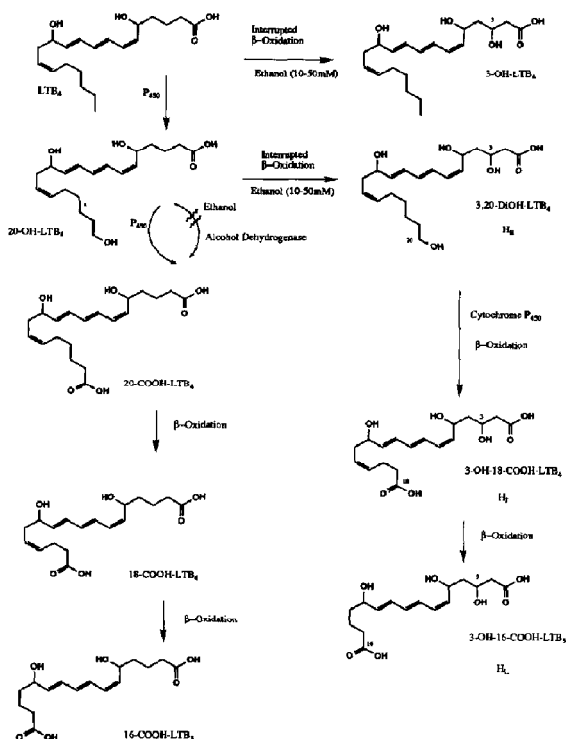

Scheme I

Scheme III

Figure 5. Summary of metabolic events occurring in isolated rat hepatocytes during metabolism of LTB$_4$ in the presence of ethanol. In the absence of ethanol, alcohol dehydrogenase is the primary route of metabolism 20-OH-LTB$_4$ to 20-COOH-LTB$_4$. The step is also catalyzed by cytochrome P450 which can catalyze this ω-oxidation step. Unique metabolites formed in the presence of alcohol have a 3-hydroxy substituent.

hydroxy group to a 3-oxo moiety by L-3-hydroxyacyl-CoA dehydrogenase would require nicotinamide adenine dinucleotide (NAD$^+$) as a cofactor, which is in short supply when ethanol is present in hepatocyte incubations [21]. There are two potential sites for LTB$_4$ β-oxidation within the hepatocyte, the peroxisome, and the mitochondria. There are some differences between the enzymes involved in β-oxidation, however, the overall steps are identical. Recently, it has been shown that both mitochondria and peroxisomes participate in hepatic LTB$_4$ metabolism [22]. For both sites of β-oxidation, LTB$_4$ must be converted into the coenzyme-A (CoA) thioester. However, for mitochondrial oxidation, formation of an acyl carnitine ester is required for transport into the mitochondria. Based upon the observed metabolism of 20-COOH-LTB$_4$ by both of these organelles, it would appear that both of these required esters can be readily synthesized when an ω-carboxyl group is present in the LTB$_4$ metabolite. Formation of LTB$_4$-CoA by rat liver microsomes has also been reported [23], but nothing has been reported for the formation of the LTB$_4$-carnitine ester. Peroxisomal β-oxidation involves fatty acyl CoA oxidase which

mediates the first step of β-oxidation with the liberation of H$_2$O$_2$ [24]. This H$_2$O$_2$ can be used in conjunction with catalase to metabolize ethanol to acetaldehyde within the peroxisome [25]. However, further oxidation within the peroxisome requires NAD$^+$, the lack of which prevents further β-oxidation. However, in the case when an ω-carboxy metabolite is formed by cytochrome P450-dependent oxidation, uptake into the mitochondria can occur. Once in the mitochondria (as the 20-COOH-CoA ester) β-oxidation might proceed because of the ability of the mitochondria to oxidize NADH to NAD$^+$. Because the reducing equivalants for ethanol metabolism in the cytosol largely come from the capacity of the malate-aspartate shuttle in the mitochondria membrane [26], the availability of NAD$^+$ within the mitochondria is somewhat reduced and overall metabolism by β-oxidation is partially inhibited. Taken together, these biochemical pathways would account for the peroxisomal formation of 3-hydroxy metabolites of LTB$_4$ with continued, but reduced, β-oxidation within the mitochondria of ω-oxidized products formed by cytochrome P450.

It is not known how the ethanol-dependent formation of 3-hydroxylated metabolites of LTB$_4$ relates to the recently reported 3-hydroxylated metabolites of dodecanoate, cis-5-decenoate, and cis-5-tetradecenoate produced from the 10,000 × g fraction from rat liver homogenates [27]. However, in incubations with the latter substrate, the authors reported that the addition of NAD$^+$ did not appear to affect the extent of 3-hydroxylated metabolite accumulation, suggesting that 3-hydroxylated fatty acid metabolites can accumulate even when NAD$^+$ is not in short supply in the 10,000 × g pellet model.

The LTB$_4$ metabolite, 3-OH-16-COOH-LTB$_3$, probably arose from 3-OH-18-COOH-LTB$_4$ by chain-shortening through β-oxidation with the CoA ester located at the C-18 carbon atom, as suggested above within the mitochondria. Because β-oxidation typically results in the loss of two carbons as methylene units, this would lead to a loss of 28 u from an homologous series of β-oxidize metabolites. However, as shown for the β-oxidation of 18-COOH-LTB$_4$ [15] and 18-COOH-N-acetyl-LTE$_4$ [28], only 26 u are lost when an isolated double bond is present an even number of carbons from the CoA ester moiety. The dicarboxylic acid metabolites of LTB$_4$ presented above indeed differed by only 26 u and formation of the reduced metabolite, 3-OH-16-COOH-LTB$_3$, strongly argues for the operation of 2,4-dienoyl-CoA reductase in the processing of the CoA ester of 3-OH-18-COOH-LTB$_4$ following oxidation by acyl-CoA dehydrogenase (mitochondrial process) [29].

Conclusion

The presence of ethanol in rat hepatocyte incubations caused an alteration of the normal metabolite profile resulting from exogenously administered LTB$_4$.

Although ethanol does inhibit LTB_4 metabolism mediated by alcohol dehydrogenase [14, 17], we found that β-oxidation can indeed take place in the presence of 44 mM ethanol. The duration of incubation and ethanol concentration used in this study were chosen to optimize production of previously unidentified LTB_4 metabolites; but it should be mentioned that these metabolites were also observed when lower ethanol concentrations were employed in hepatocyte incubations. Further investigations of mechanisms of metabolite formation and possible involvement of these metabolites in alcohol-related pathophysiology are currently in progress.

Acknowledgment

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