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Defective Degradation of Leukotrienes in Peroxisomal-Deficient Human Hepatocytes

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Chain-shortening via β-oxidation from the ω-end has been recognized as the major pathway for the degradation of the biologically active cysteinyl leukotrienes as well as LTB_4. The metabolic compartmentation of this pathway was investigated in peroxisomal-deficient (Zellweger syndrome) and normal human hepatocytes. Leukotriene metabolism was studied in isolated hepatocytes by incubation with ω-carboxy-[^{3}H]LTE_4 as well as ω-carboxy-[^{3}H]LTB_4. Analysis was done by HPLC, UV-detection and radioactivity measurements. Incubation of normal hepatocytes with ω-carboxy-[^{3}H]LTE_4 or ω-carboxy-[^{3}H]LTB_4 resulted in the formation of the corresponding β-oxidation products, whereas β-oxidation derivatives were not detected as products formed by peroxisome-deficient human hepatocytes. These results underline the essential contribution of peroxisomes in the catabolism and inactivation of cysteinyl leukotrienes and LTB_4 in humans.

The metabolic inactivation (1) and degradation of leukotrienes (2-4) is of major importance because of the biological potency of these lipid mediators. Both the chemotactically active LTB_4 and the cysteinyl leukotrienes which are involved in inflammatory and hypersensitivity reactions are generated under various pathophysiological conditions (5-8). The liver is the major organ for uptake, catabolism, and biliary elimination of leukotrienes (9-11). Chain shortening via β-oxidation from the ω-end has been recognized as an important pathway for the degradation of the cysteinyl leukotriene LTE_4 as well as LTB_4 (12).

Recently, the metabolic compartmentation of this pathway was studied in normal and clofibrate-treated rat liver peroxisomes (4). Isolated hepatocytes from rats pretreated with the peroxisome proliferator clofibrate produced highly increased amounts of β-oxidation products derived from ω-carboxy-LTB_4 and ω-carboxy-N-acetyl-LTE_4 as compared to control hepatocytes. Furthermore, ω-carboxy-LTB_4 was oxidized both in isolated rat liver peroxisomes and mitochondria, whereas ω-carboxy-N-acetyl-LTE_4 was exclusively inactivated in peroxisomes (4). These results in addition with the findings of an altered pattern of urinary leukotriene metabolites in patients with an inherited peroxisome deficiency disorder (Zellweger syndrome) suggested that peroxisomes may play an important role in the β-oxidation of leukotrienes (13).

In the present study the contribution of peroxisomes to leukotriene degradation and inactivation was investigated in isolated peroxisome-deficient human hepatocytes showing a normal mitochondrial β-oxidation capacity.

MATERIALS AND METHODS

Chemicals. [5,6,8,9,11,12,14,15-$^3H_8$]LTE_4 (4.8 TBq/mmol) and [5,6,8,9,11,12,14,15-$^3H_8$]LTB_4 (7.0 TBq/mmol) were obtained from Du Pont-New England Nuclear. Unlabeled ω-carboxy-LTE_4, ω-carboxy-dinor-LTE_4, ω-carboxy-tet-

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Abbreviations: HTMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*(1)-oxyl; LT, leukotriene; RP-HPLC, reversed-phase high-performance liquid chromatography; VLCFAs, very long chain fatty acids.

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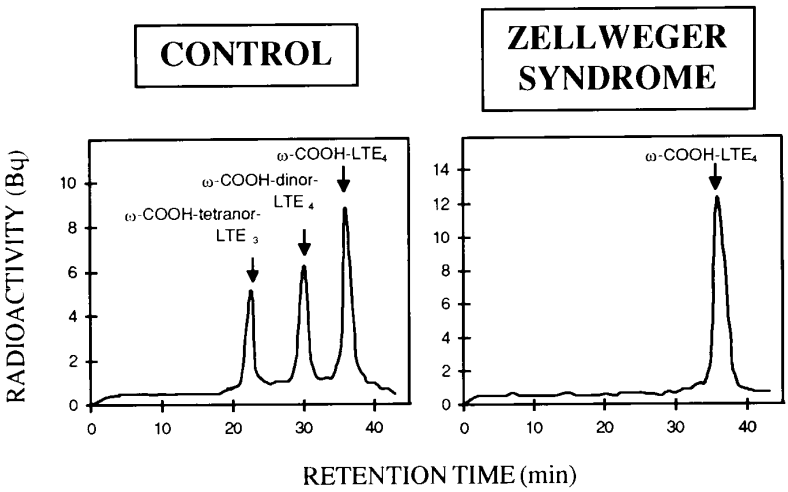

FIG. 1. Incubation of normal and peroxisomal-deficient (Zellweger syndrome) human hepatocyte suspensions with ω-carboxy-[^{3}H]LTE$_4$.

ranor-LTE$_4$, ω-carboxy-LTB$_4$ and ω-carboxy-dinor-LTB$_4$ were purchased from Cascade Biochem Ltd. (University of Reading, United Kingdom). HTMP was obtained from Sigma Chemical Co., St. Louis, MO. All other substances were obtained from commercial sources at the highest purity available.

ω-Carboxy-(^{3}H)LTE$_4$ and ω-carboxy-(^{3}H)LTB$_4$ were prepared from [^{3}H]LTE$_4$ and [^{3}H]LTB$_4$, respectively, using rat liver microsomes and cytosol as described (14,15).

Patients. Leukotriene metabolism was studied in hepatocytes obtained from a peroxisomal-deficient patient and a control patient. The patient with peroxisome deficiency disorder exhibited the characteristic clinical and biochemical abnormalities described for Zellweger syndrome (16). Specific biochemical analyses included VLCFAs ($>C_{22}$) in plasma and fibroblasts as well as plasma bile acid intermediates and *de novo* plasmalogen biosynthesis in cultured fibroblasts. Mitochondrial β-oxidation activity was assayed in cultured fibroblasts using [^{14}C]palmitic acid (4 μM, Amersham Corp) and found to be in the range of normal cell lines. The control subject showed a normal peroxisomal and mitochondrial β-oxidation capacity.

Cell isolation and culture. Hepatocytes were prepared from liver biopsies (17). After washing, the cells were stored

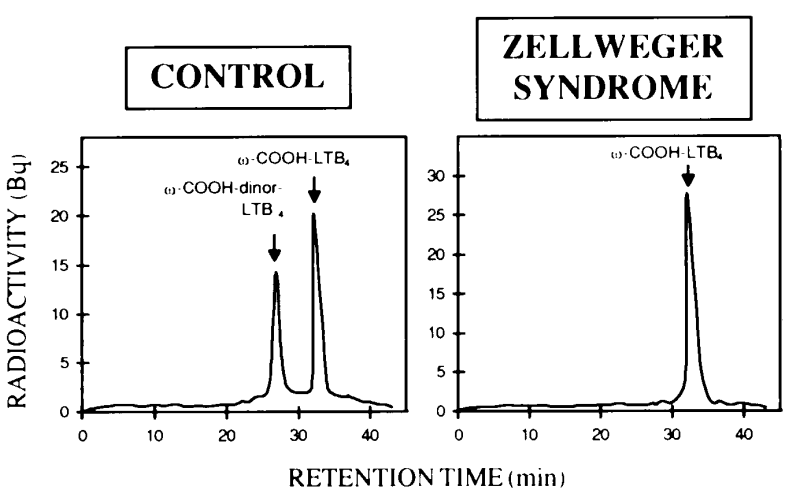

FIG. 2. Incubation of normal and peroxisomal-deficient (Zellweger syndrome) human hepatocyte suspensions with ω-carboxy-[^{3}H]LTB$_4$.

on ice in modified Krebs/Henseleit bicarbonate buffer containing 118 mM NaCl, 4.7 mM KCl, 0.6 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 25 mM $NaHCO_3$, 5.5 mM glucose, 1,2 mM $MgCl_2$ and 1,3 mM $CaCl_2$ (pH 7,4). Viability of the hepatocytes was >65% as juged from trypan blue exclusion. Contamination with non-parenchymal cells was less than 2% as determined by light microscopy. Hepatocytes were plated on 35-mm tissue-culture dishes (1.5×10^6 cells/dish) in 2 ml Ham's F12 medium (Boehringer Mannheim, Mannheim, Germany) containing 10% fetal bovine serum and 20 U/l insulin. Cultures were maintained at 37°C in the presence of 95% air and 5% CO_2; 4h after plating, the culture medium was changed to the Krebs/Henseleit incubation buffer (500 μl/dish).

Leukotriene metabolism in hepatocytes. Hepatocyte suspensions (3×10^9 cells/l) were incubated with the [^{3}H]-leukotrienes (10 nM) in incubation buffer at 37°C in the presence of 95% air and 5% CO_2. After 1h the incubations were stopped by addition of 4 vol ice-cold methanol containing 1 mM HTMP.

Separation and analysis of leukotriene metabolites. Precipated proteins from samples containing 80% methanol were removed by centrifugation. The samples were dried, redissolved in 30% aqueous methanol. Separation by RP-HPLC was performed on a C_{18} Hypersil column (4.6 mm $\times$ 250 mm, 5-μm particle size; Shandon, Runcorn, UK). Leukotriene metabolites were separated using a system containing 100% water, switching at 5 min to a linear gradient of 40-80% aqueous methanol within 35 min, followed by 10 min of aqueous methanol and a linear gradient of 80-100% aqueous methanol within 5 min, and additionally by 20 min of 100% methanol. All solvents contained 0.1% acetic acid and the pH was adjusted to 5.0 with ammonium hydroxide. The HPLC eluate was either measured continously for tritium radioactivity using a liquid scintillation device (Raytest, Straubenhardt, Germany) or sampled in 0.5-min fractions using a fraction collector (LKB, Bromma, Sweden). Radioactivity was measured in aliquots of the HPLC fractions in a β-scintillation counter (Beckman Instruments, Fullerton, CA, USA). Absorbance of unlabeled leukotriene standards was followed at 280 nm for LTE_4 metabolites and at 270 nm for LTB_4 metabolites.

The identity of each leukotriene metabolite was confirmed by its co-chromatography with adequate synthetic standards in a mobile-phase system forming a methanol gradient and re-chromatography of each metabolite before and after N-acetylation in an appropriate solvent system (18). Retention times of the standards are indicated in the figures by arrows.

RESULTS AND DISCUSSION

The results of this study show that normal human hepatocytes β-oxidize ω-carboxy-[^{3}H]LTB_4 as well as the cysteinyl leukotriene metabolite ω-carboxy-[^{3}H]LTE_4 from the ω-end (Figs. 1 & 2). Exposure of normal hepatocyte suspensions to ω-carboxy-[^{3}H]LTE_4 or ω-carboxy-[^{3}H]LTB_4 led to the formation of the corresponding β-oxidation products. These included ω-carboxy-dinor-[^{3}H]LTE_4, ω-carboxy-tetranor-[^{3}H]LTE_3 and ω-carboxy-dinor-[^{3}H]LTB_4 (Figs. 1 & 2).

Volatile radioactivity was not present. N-acetylated derivatives of ω-carboxy-[^{3}H]LTE_4 were not detected as products formed by human hepatocytes. This is in agreement with previous findings suggesting that intracellular N-acetylation of LTE_4 and presumably further ω-oxidation products derived from LTE_4 proceeds in the kidneys (18).

In contrast to normal hepatocytes incubation of peroxisomal-deficient hepatocytes with ω-carboxy-[^{3}H]LTE_4 as well as ω-carboxy-[^{3}H]LTB_4 failed to result in the formation of any β-oxidation product indicating that these metabolites were not further degraded by human peroxisomal-deficient hepatocytes (Figs. 1 & 2).

Previous *in vitro* experiments indicate that the degradation of LTB_4 can also proceed in rat liver mitochondria (4,19). It is unlikely, however, that the mitochondrial β-oxidation of ω-carboxy-LTB_4 plays a major role in human hepatocytes since in all incubation assays [^{14}C]-palmitic acid was catabolized under the same conditions in normal as well as peroxisomal-deficient hepatocyte suspensions indicating a functional mitochondrial β-oxidation capacity.

Our results demonstrate that in hepatocytes obtained from a patient with Zellweger syndrome, an inherited disease of peroxisomal bio-genesis, inactivation and degradation of leukotrienes is highly impaired. These findings in peroxisomal-deficient hepatocytes provide direct evidence for an exclusive degradation of cysteinyl leukotrienes and LTB_4 in peroxisomes and underline the essential role of peroxisomes in the catabolism and inactivation of leukotrienes in humans.

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