Metabolism of Prostaglandin F_{2α} in Zellweger Syndrome

Peroxisomal β-Oxidation Is of Major Importance for In Vivo Degradation of Prostaglandins in Humans

Ulf Diczfalussy,* Bengt Frode Kase,* Stefan E. H. Alexson,[†] and Ingemar Björkhem*

*Department of Clinical Chemistry I, Huddinge University Hospital, S-141 86 Sweden; †Department of Pediatric Research, Rikshospitalet, The National Hospital, N-0027 Oslo, Norway; and ‡Department of Metabolic Research, The Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

Abstract

We have recently shown in vitro that the peroxisomal fraction of a rat liver homogenate has the highest capacity to β-oxidize prostaglandins. In order to evaluate the relative importance of peroxisomes for this conversion also in vivo, we administered [³H]prostaglandin F_{2α} to an infant suffering from Zellweger syndrome, a congenital disorder characterized by the absence of intact peroxisomes. As a control, labeled compound was administered to two healthy volunteers. Urine was collected, fractionated on a SEP-PAK C₁₈ cartridge, and subjected to reversed-phase high-performance liquid chromatography. The Zellweger patient was found to excrete prostaglandin metabolites considerably less polar than those of the control subjects. The major urinary metabolite in the control subjects was practically absent in the urine from the Zellweger patient. The major urinary prostaglandin F_{2α} metabolite from the Zellweger patient was identified as an ω-oxidized C₂₀-prostaglandin, 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid. The major urinary prostaglandin F_{2α} metabolite from the control subjects had chromatographic properties of a tetranol (C₁₆) prostaglandin, in accordance with earlier published data. The present results, in combination with our previous in vitro data, indicate that peroxisomal β-oxidation is of major importance for in vivo chain shortening of prostaglandins. (*J. Clin. Invest.* 1991; 88:978–984.) Key words: Cerebro-hepato-renal syndrome • chain shortening • ω-oxidation

Introduction

Prostaglandins are very rapidly metabolized in vivo. In humans, the methyl end side chain is first modified, leading to formation of the major circulating metabolites, 15-keto-13,14-dihydro prostaglandins (1, 2). Further ω- and β-oxidation leads to chain-shortened products that are excreted in the urine (3–5). The majority of prostaglandin metabolites are thus C₁₈ or C₁₆ compounds, the result of one or two cycles of β-oxidation. It has been reported that prostaglandins may be β-oxidized in mitochondria in a carnitine-dependent process (6, 7). Recently it was shown that also phthalate- or clofibrate-induced rat liver peroxisomes have a high capacity to chain-shorten prostaglan-

dins (8–10), possibly considerably higher than that of the mitochondrial fraction (9).

In spite of the fact that peroxisomes have a high capacity to β-oxidize prostaglandins in vitro, it is difficult to evaluate the relative role of this organelle under conditions in vivo.

Patients with the Zellweger syndrome, who therefore lack peroxisomes, may offer a unique possibility to evaluate the relative importance of peroxisomes for prostaglandin degradation. In the present investigation, a girl with this rare autosomal recessive disorder was given radioactive prostaglandin F_{2α}. The pattern of labeled urinary metabolites of the infant was compared with that of two healthy adults.

Methods

Case history

The infant (I.H.) was a first-born full-term female of healthy nonrelated parents. She was born with a moderate asphyxia and weighed 3,070 g. At birth she presented severe hypotonia with areflexia, large fontanelle and metopic suture, facial dysmorphic patterns as described for Zellweger syndrome, and congenital glaucoma. On the second day of life phenobarbital (3–5 mg/kg body wt) was instituted to treat seizures. Hepatomegaly became apparent from 1 mo of age. Increasing levels of serum transaminases were detected from birth but no signs of bile stasis or liver failure were found. Her nuchal hypotonia persisted, her seizures became refractory, and she had severe developmental delay. The electroencephalograms were abnormal with spike foci and sharp wave discharges. In addition, brushes and asynchrony pointed to immaturity of the brain as seen in premature birth infants. No electroretinographic or brain stem auditory evoked response signals could be detected. Serum bile acids had the following levels: cholic acid 3.14 μmol/liter (4.18±2.31 μmol/liter [11]), chenodeoxycholic acid 3.96 μmol/liter (7.09±3.01 [11]), trihydroxycoprostanic acid 24.6 μmol/liter (normally undetected levels), C₂₉-trihydroxy-dicarboxylic-coprostanic acid: trace amounts (normally undetected levels) (12). Plasmalogen synthesis activity was severely reduced. Synthesis rate was measured by Moser using the double isotope technique described by Roscher et al. (13). The mean ³H/¹⁴C was 5.01 (normal controls 0.67±0.19, "pseudo Zellweger" 0.83±0.21). There was no diagnostic or therapeutic indication for liver biopsy. She died at the age of 4 mo after an episode of apnea with bradycardia. Bile acids in a postmortem blood sample had the following levels: cholic acid 0.74 μmol/liter, chenodeoxycholic acid 0.86 μmol/liter, trihydroxycoprostanic acid 7.9 μmol/liter, C₂₉-trihydroxy-dicarboxylic-coprostanic acid 3.9 μmol/liter.

In accord with the parents' wish, no autopsy was performed.

Chemicals

Human albumin (20%) was obtained from KabiVitrum AB, Stockholm, Sweden. [9-³H] Prostaglandin F_{2α}, 16 Ci/mmol, was purchased from New England Nuclear, Dreieich, FRG. 20-Hydroxy prostaglandin F_{2α} and 20-hydroxy-13,14-dihydro-15-keto-prostaglandin F_{2α} were isolated from incubations of prostaglandin F_{2α} with lung microsomes or lung homogenates from pregnant rabbits, as described earlier (14, 15).

Address reprint requests to Dr. Diczfalussy, Department of Clinical Chemistry I, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

Received for publication 3 January 1991 and in revised form 25 April 1991.

J. Clin. Invest.

© The American Society for Clinical Investigation, Inc.

0021-9738/91/09/0978/07 \$2.00

Volume 88, September 1991, 978–984

9,11-Dihydroxy-15-oxoprost-5-ene-1,20-dioic acid was prepared by incubating 100 μ g of 20-hydroxy-13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ with 3 ml 20% rat liver cytosol (0.1 M Tris, pH 8.5) supplemented with 3 mg NAD^+ at 37°C for 30 min. The reaction mixture was extracted, treated with ethereal diazomethane, and analyzed by reversed-phase high performance liquid chromatography (RP HPLC). The RP HPLC chromatogram (see Fig. 3 B) showed three peaks that were further identified by gas chromatography-mass spectrometry. Peak I and II (as trimethylsilyl ether derivatives) had mass spectra identical to the spectrum of 20-hydroxy-13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (15). Peak II is probably the hemiketal form of peak I. Peak III had a spectrum identical to that reported for 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid (as its trimethylsilyl ether derivative) (15).

Extraction of urine

20 ml of urine was acidified to pH 3 with 1 N HCl and applied to a SEP-PAK C_{18} cartridge. The cartridge was eluted with 5-ml portions of water, 15% ethanol in water, petroleum ether, methyl formate, and methanol (16). The recovery of radioactivity was 94.6–103%. The solvent was evaporated under a gentle stream of nitrogen. Samples were dissolved in a small volume of methanol for analysis by HPLC.

Reversed-phase HPLC of SEP-PAK C_{18} eluates

Analytical HPLC of the methyl formate fraction was carried out on a Waters Associates (Milford, MA) Nova Pak C_{18} 4- μ m column (Radial-Pak, 8 mm $\times$ 10 cm) using methanol/water/acetic acid 55:45:0.01 vol/vol as mobile phase. The flow rate was 1.25 ml/min. The HPLC system was connected to a FLOW ONE/ β model IC radioactivity detector (Radiomatic Instruments & Chemical Co. Inc., Tampa, FL) equipped with a 2.5-ml flow cell. Preparative HPLC of the methyl formate fraction was performed on a Polygosil 60–10- μ m C_{18} column (Macherey-Nagel, Dürren, FRG), 10 mm $\times$ 50 cm, using methanol/water/acetic acid 60:40:0.01 vol/vol as mobile phase with a flow rate of 4.0 ml/min.

The more polar SEP-PAK C_{18} -fraction (15% ethanol in water) was analyzed using the Nova Pak C_{18} column with methanol/water/acetic acid 40:60:0.01 as mobile phase (flow rate 1.0 ml/min).

Straight-phase HPLC

The SEP-PAK C_{18} methyl formate eluates from the control subjects were analyzed by straight-phase HPLC using a Waters Associates Resolve 5- μ m column (8 mm $\times$ 10 cm). The mobile phase was hexane/ethanol/acetic acid 75:25:0.01 vol/vol and the flow rate 1.0 ml/min.

Radio gas-liquid chromatography

Gas chromatography with radioactivity detection was performed on a Pye Unicam model 204 gas chromatograph connected to an ESI nuclear radioactivity detector (Klempfer House, Reigate, Surrey, England). The column was a 3-m $\times$ 3-mm i.d. glass tubing packed with 1% OV-1 on Chromosorb 750, 60–80 mesh. Retention times were converted to C values (relative retention times) using saturated fatty acid methyl esters as reference compounds (17).

Gas chromatography-mass spectrometry

Mass spectrometric analyses were performed using either an LKB 2091 gas chromatograph-mass spectrometer equipped with a 30-m $\times$ 0.25-mm DB 1301 column (J&W Scientific, Folsom, CA) or a Hewlett-Packard Co. (Palo Alto, CA) model 5890 gas chromatograph (equipped with a 13-m $\times$ 0.18-mm DB-5 column, J&W Scientific)/model 5970 mass selective detector.

Radioactivity in urine and chromatographic fractions was analyzed in a model 1217 Rackbeta liquid scintillation counter (LKB Produkter, Bromma, Sweden).

Administration of radiolabeled prostaglandin $F_{2\alpha}$

25 μ Ci of prostaglandin $F_{2\alpha}$ [$^3H(N)$] (16.0 Ci/mmol) was dissolved in 50 μ l of methanol and purified on a Waters Associates Radial Pak

Nova Pak C_{18} -column (8 mm $\times$ 10 cm). The mobile phase was methanol/water/acetic acid 60:40:0.01 vol/vol and the flow rate was 1.0 ml/min. 60 fractions of 0.5 ml were collected. Two μ l of each fraction was analyzed for radioactivity. Fractions corresponding to prostaglandin $F_{2\alpha}$ were pooled and taken to dryness under a gentle stream of nitrogen. The pooled material was dissolved in 700 μ l of ethanol. Two aliquots of 3 μ l each were removed for analysis on HPLC and radioactivity determination by liquid scintillation counting. The rest was subjected to sterile filtration. 12 μ Ci of [3H]prostaglandin $F_{2\alpha}$ (0.3 μ g) was mixed with 4.5 ml of sterile 20% human albumin solution and injected i.v. (0.5 ml/min) into the patient or the control subjects. Urine from the infant was collected by permanent urethral catheterization for a 70-h period after the infusion.

Derivatization for gas chromatographic analysis

Carboxyl groups were converted to methyl- or ethyl esters by treatment with ethereal diazomethane or diazoethane, respectively. Some metabolites were reacted with *O*-methoxyamine hydrochloride in pyridine to produce methyl oxime derivatives from keto groups. Hydroxyl groups were derivatized into trimethylsilyl ethers by treatment with trimethylchlorosilane-hexamethyl-disilazane-pyridine 1:2:3 (vol/vol/vol).

β -Oxidation of [$1-^{14}C$]palmitic acid in cultured fibroblasts from the infant suffering from Zellweger syndrome and from a healthy age-matched infant

Tissue cultures. Skin biopsies were taken from the forearm. Cultures of skin fibroblasts were routinely grown in basal Eagle's medium with 16% (vol/vol) fetal calf serum. Penicillin (100 U/ml), streptomycin (100 μ g/ml), amphotericin (2 U/ml), and L-glutamine (1.5 μ mol/ml) were added to the medium. Monolayer cultures were established in 75-cm² flasks with 21 ml of medium at 37°C, gassed with 5% CO₂ in air.

Incubations, extractions, and chromatographic procedures. A mixture of labeled and unlabeled palmitic acid dissolved in acetone was slowly mixed into essentially fatty acid free bovine serum albumin, 3% (wt/vol) under a gentle stream of N₂ at room temperature. The 2 mM palmitate solution was sterilized by filtration through a filter (0.22 μ m; μ Star, Costar Corp., Cambridge, MA). The incubations were initiated by adding the substrate (90 nmol) directly to the medium in the flasks with cells growing in monolayer. The medium used in these incubations consisted of basal Eagle's medium enriched with 1.6% fetal calf serum. Blank incubations were performed using cell cultures in the same medium that had been boiled for 5 min before addition of substrate. The incubations were terminated by turning the flasks upside down, separating the medium from the cells. 4 ml of 0.1 M sulfuric acid was added to the medium. The liberated ¹⁴CO₂ was trapped in a small well with a piece of filter paper soaked with 300 μ l of methanol/diphenylethylamine (1:1, vol/vol). After trapping of the ¹⁴CO₂ at room temperature over night, the medium was removed and the monolayer was washed twice with 0.9% NaCl before harvesting with a Costar disposable cell scraper. The flasks were washed with 0.9% NaCl and ethanol. A batch of the cell sap was collected for protein measurement, before the cell sap and the medium were mixed. The mixture was extracted with a SEP-PAK C_{18} cartridge, and the remaining ¹⁴C activity in the medium was regarded as ¹⁴C-labeled acetic acid. Blank activities of ¹⁴CO₂ and water-soluble ¹⁴C activity were subtracted. Radioactivity was counted in a Packard Instruments Co. (Meriden, CT) model 2420 Tri-Carb liquid scintillation counter after addition of counting solution (Packard Opti-Fluor).

Ethical aspects. The study was approved by the Ethical Committee (Region II) of Rikshospitalet, Oslo, Norway and by the Ethical Committee of Huddinge University Hospital, Karolinska Institutet, Sweden.

Results

The appearance of radioactive metabolites in urine after injection of ³H-labeled prostaglandin $F_{2\alpha}$ into an infant with Zell-

weger syndrome and two control subjects is shown in Fig. 1. There was a rapid excretion of radioactive metabolites in the urine from all subjects. The main part of the recovered radioactivity in the urine was excreted within the first 5 h in all subjects. The urine samples were extracted by means of a SEP-PAK C₁₈ cartridge. A fraction containing polar metabolites was collected (eluted with 15% ethanol in H₂O) followed by a neutral fraction (eluted with petroleum ether) and a fraction containing both prostaglandins and relatively nonpolar metabolites (eluted with methyl formate) (16). The distribution of radioactivity between the different eluates from the SEP-PAK C₁₈ cartridge is shown in Table I for the urine samples collected 3 and 2.25 h after injection (control subjects) and 2 h after injection (Zellweger). About 65% of the radioactivity from the control subjects was recovered in the 15% ethanol eluate whereas only 1.6% of the radioactivity from the infant was found in this fraction. In the analysis of the urine from the infant, 96% of the radioactivity was eluted in the methyl formate fraction. The fraction of radioactivity in the methyl formate eluate decreased somewhat in urine samples collected at later intervals after injection in the Zellweger patient (84.6% in the urine collected 3–9 h after administration). It is evident that the metabolites recovered from the control subjects were considerably more polar than those from the patient.

The methyl formate fractions from the control subjects and the infant with the Zellweger syndrome were subjected to HPLC. The chromatograms from one of the two control subjects (female) and the Zellweger patient are shown in Fig. 2. The chromatogram for the Zellweger patient showed five peaks that eluted later (i.e., were less polar) than the single peak obtained from the control subject in the same system. Materials corresponding to peaks 1, 2, and 3 in Fig. 2 were isolated by reversed phase HPLC. The compound corresponding to peak 1 had a retention time of 6.40 min. The reference compounds 20-hydroxy prostaglandin F_{2α} and 20-hydroxy-13,14-dihydro-15-keto prostaglandin F_{2α} had retention times of 5.50 and 6.80 min, respectively. The material corresponding to peak 1 (Fig. 2) was treated with ethereal diazomethane and the product was analyzed by RP HPLC. The HPLC chromatogram, Fig. 3 A,

Figure 1. Urinary excretion of tritium-labeled products after injection of [9-³H(N)]prostaglandin F_{2α} into a patient with the Zellweger syndrome and two healthy control subjects.

Table I. Solid Phase Extraction of Urine from Two Control Subjects and a Patient with Zellweger Syndrome after Intravenous Administration of Tritium-labeled Prostaglandin F_{2α}

SEP-PAK C ₁₈ eluate	Percentage of recovered radioactivity		
	Control* (female)	Control† (male)	Zellweger‡ (female)
Water	0.3	1.1	0.2
15% ethanol	64.1	64.0	1.6
Petroleum ether	0	0	0
Methyl formate	35.0	34.1	96.0
Methanol	0.7	0.8	2.2

* Urine collected 1–3 h after administration of [³H]prostaglandin F_{2α}.

† Urine collected 1–2.25 h after administration of [³H]prostaglandin F_{2α}. ‡ Urine collected 0–2 h after administration of [³H]prostaglandin F_{2α}.

shows that the diazomethane-treated material resolved into three peaks. Fig. 3 B shows the HPLC chromatogram of the methyl esters of a reference mixture containing 20-hydroxy-13,14-dihydro-15-keto prostaglandin F_{2α} (peak I and II) and 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid (peak III). As can be seen in Fig. 3, the three peaks of the material from the Zellweger patient (Fig. 3 A) had retention times identical to those of the three peaks of the reference mixture (Fig. 3 B).

The material corresponding to peak 1 (Fig. 2) was also analyzed by radio gas-liquid chromatography. The methyl ester-trimethylsilyl ether-methyl oxime derivative had a C value of

Figure 2. Reversed-phase HPLC chromatograms of methyl formate eluates from a SEP-PAK C₁₈ fractionation of urine sampled after infusion of tritium-labeled prostaglandin F_{2α} to a Zellweger patient (upper panel) and a healthy control subject (lower panel). Flow rate: 1.25 ml/min.

Figure 3. Reversed-phase HPLC chromatograms of methyl esters of peak 1 in Fig. 2. (A) and a reference mixture containing 20-hydroxy-13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (I and II) and 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid (III) (B).

27.1, and the corresponding ethyl ester derivative had a C value of 28.1. The difference in C value between the methyl and ethyl ester is in accord with the contention that the major part of the material is a dicarboxylic acid.

In order to identify the dicarboxylic acid also by combined gas chromatography-mass spectrometry, more endogenous material was required. Urine (130 ml) from the Zellweger patient was extracted and fractionated by HPLC. The gas chromatographic retention time of the major metabolite, peak 1 in Fig. 2, (as methyl ester-trimethyl silyl ether derivative) was found to be identical to that of the reference compound 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid. The mass spectrum showed major ions at m/z 466 (M-90), 451, 435, 376, 325, 308, 295, 255, 233, and 218 as earlier reported for 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid (methyl ester-trimethylsilyl ether) (15).

The content of peak 2 in Fig. 2 was converted to methyl ester-methyloxime-trimethylsilyl ether and analyzed by radio gas-liquid chromatography. One major component with a C value of 24.2 appeared together with minor peaks with C values of 25.0, 25.8, and 27.3.

The methyl ester-methyloxime-trimethylsilyl ether of the material in peak 3 (Fig. 2) was resolved into four components

by radio gas-liquid chromatography with C values of 23.0, 24.8, 25.7, and 26.5.

Peaks 4 and 5 in Fig. 2 had retention times similar to those of prostaglandin $F_{2\alpha}$ and 15-keto-13,14-dihydro prostaglandin $F_{2\alpha}$, respectively. We were not able to make a positive identification, however, owing to the very small amount of endogenous material available.

The material in peak 6 in Fig. 2 was subjected to straight-phase HPLC and found to be a complex mixture. No further attempts were made to characterize this peak.

The 15% ethanol eluate from the control subjects were subjected to reversed phase HPLC. The HPLC chromatogram from one of the control subjects is shown in Fig. 4. The material was resolved into four peaks. The major peak had a retention time of 6.4 min and constituted 54.1% of the applied radioactivity (corresponding to 34.6% of the radioactivity in the urine sample). The material corresponding to this peak was isolated by HPLC and derivatized for gas chromatography. One major radioactive component, with a C value of 23.8 (methyl ester-methyloxime-trimethylsilyl ether) was detected by radio gas-liquid chromatography. This component constituted the major urinary metabolite in the control subject. According to the chromatographic properties and the C value, this component is most probably identical to 5 α ,7 α -dihydroxy-11-keto-tetranorprosta-1,16-dioic acid which has been reported to be the major urinary metabolite of prostaglandin $F_{2\alpha}$ in the human (3).

Fibroblasts were isolated from the Zellweger patient and from an age-matched healthy control in order to investigate whether the mitochondrial β -oxidation system was operating normally in the Zellweger patient. Monolayer cultures of the two cell lines were established and incubated with [1- 14 C]palmitic acid. β -Oxidation activity was determined as described in Methods. The β -oxidation activity determined in fibroblasts (measured in triplicate) from the Zellweger patient and an age-matched control subject is shown in Table II. There was no significant difference in specific β -oxidation activity between the Zellweger fibroblasts and the control fibroblasts suggesting a normal mitochondrial β -oxidation activity in the Zellweger fibroblasts.

Figure 4. Reversed-phase HPLC chromatogram of a 15% ethanol eluate from a SEP-PAK C_{18} fractionation of urine from a healthy control subject who had been injected with tritium-labeled prostaglandin $F_{2\alpha}$. Flow rate: 1.0 ml/min.

Table II. β -Oxidation of [1- 14 C]Palmitic Acid in Cultured Fibroblasts from an Infant Suffering from Zellweger Syndrome and from a Healthy Age-matched Infant

Sample No.	Origin	Specific β -oxidation activity
		nmol/mg protein per d
1	Control	5.77
2	Control	6.75
3	Control	5.22
4	Zellweger	6.66
5	Zellweger	9.00
6	Zellweger	9.10

Discussion

To evaluate the importance of peroxisomes in relation to mitochondria in the oxidative chain-shortening of prostaglandins, a radiolabeled prostaglandin was administered to a patient with an inherited peroxisome deficiency syndrome (Zellweger syndrome). The high levels of C_{29} -dicarboxylic acid in serum and the defect in plasmalogen synthesis in cultured fibroblasts were considered sufficient to confirm the diagnosis of Zellweger syndrome and exclude a diagnosis of "pseudo-Zellweger" syndrome. This latter syndrome (peroxisomal 3-oxoacyl-coenzyme A-thiolase deficiency) has all the clinical and pathological features of Zellweger syndrome. Accumulation of very long-chain fatty acids as well as coprostanic acidemia are found but, in the liver, peroxisomes are found to be abundant (18). The syndromes may be distinguished biochemically by a normal plasmalogen biosynthesis and no trace of C_{29} -dicarboxylic bile acid in the Pseudo-Zellweger syndrome (19).

Prostaglandin $F_{2\alpha}$ was chosen on the basis of its relatively high stability and well characterized metabolism in man (2, 3, 4, 20-23). The radioactive prostaglandin used had the tritium label in the 9 β -position. A prostaglandin 9-keto reductase has been found in rat tissues (24) leading to small losses (10%) of 3H from [9 β - 3H] prostaglandin $F_{2\alpha}$ when injected into rats (25). In monkeys (26) and in humans (3), however, the label seems to be stable and thus suitable for metabolic studies. In order to evaluate the relative importance of peroxisomes for prostaglandin oxidation, it must be assumed that the infant studied has a normal mitochondrial function. A reduced mitochondrial electron transport has been reported in some patients suffering from the Zellweger syndrome (27). However, the mitochondrial 26-hydroxylase, a monooxygenase, converting the bile acid precursor 5 β -cholestane-3 α ,7 α ,12 α -triol into 5 β -cholestane-3 α ,7 α ,12 α ,26-tetrol was found to function normally in such patients (28). Very long chain fatty acids and trihydroxycoprostanic acid, which are oxidized in peroxisomes, accumulate in patients with the Zellweger syndrome (28, 29). Long chain fatty acids which are mainly oxidized in mitochondria are not accumulated, however. The latter finding supports the contention that the mitochondrial β -oxidation capacity is not reduced in Zellweger patients. In the present study, mitochondrial β -oxidation activity was assayed in fibroblasts isolated from the Zellweger patient and from an age-matched healthy

infant. As shown in Table II the specific β -oxidation activity was not different in the fibroblasts from the Zellweger patient as compared to the fibroblasts from the control subject, indicating a normal mitochondrial β -oxidation activity.

There was a rapid excretion of radioactive metabolites in the urine after administration of [3H]prostaglandin $F_{2\alpha}$, as can be seen in Fig. 1. The excretion rate was similar in the Zellweger patient and the control subjects and was also similar to those reported (3, 5, 30). There was a pronounced difference in the polarity of excreted radioactive metabolites from the infant as compared to the control subjects (Table I). The ethanol eluate from the SEP-PAK C_{18} cartridge contained the major urinary metabolite in the control subjects, with a C value of 23.8 (methyl ester-methyl oxime-trimethylsilyl ether). Most probably this metabolite is 5 α ,7 α -dihydroxy-11-keto-tetranorprosta-1,16-dioic acid. The latter compound has been reported to be the major urinary metabolite of prostaglandin $F_{2\alpha}$ in humans and to have a C value of 23.8 for the methyl ester-methyl oxime-trimethylsilyl ether derivative (3). This metabolite, which has been chain-shortened by four carbons through two cycles of β -oxidation and further ω -oxidized to a dicarboxylic acid, is considerably more polar than prostaglandin $F_{2\alpha}$ itself. The less polar metabolites appearing in the methyl formate eluates, were very different in the material from the Zellweger patient and the control subjects. As can be seen in Fig. 2 almost all radioactivity from the Zellweger patient eluted later (i.e., was less polar) than the radioactivity from a control subject. It is thus evident that the prostaglandin $F_{2\alpha}$ metabolites from the Zellweger patient had been oxidized considerably less than the metabolites from the control subject.

The major urinary metabolite of prostaglandin $F_{2\alpha}$ from the Zellweger patient was characterized by HPLC and radio gas-liquid chromatography. Radio gas-liquid chromatography of peak 1 (Fig. 2) as either methyl ester-trimethylsilyl ether-methyl oxime or the corresponding ethyl ester derivative revealed a difference in C value between the two derivatives of 1 C-unit, which indicated the presence of two carboxyl groups in the molecule. The high C values (around C-27) are characteristic for ω -hydroxylated or ω -carboxy C_{20} -prostaglandins (15). The difference in C values between the methyl ester-trimethylsilyl ether derivative and the methyl ester-trimethylsilyl ether-methyl oxime derivative of peak 1 (Fig. 2) indicated the presence of a keto function in the molecule, tentatively located at C_{15} (the C values for the corresponding derivatives of 20-hydroxy-13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ were 27.4 and 27.2, respectively [15]). As shown in Fig. 3, the major metabolite from the Zellweger patient cochromatographed with 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid (as methyl esters). Mass spectrometry of the major prostaglandin $F_{2\alpha}$ metabolite from the Zellweger patient (as methyl ester-trimethylsilyl ether derivative) confirmed that the structure was in fact 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid. It may thus be concluded that the major metabolite of prostaglandin $F_{2\alpha}$ in the Zellweger patient is a dicarboxylic acid which has not been chain-shortened and the proposed pathway for its formation is outlined in Fig. 5. The Zellweger patient is thus capable of ω -oxidation but not β -oxidation of the prostaglandin. This situation is similar to that occurring in connection with degradation of the side chain of cholesterol. Also here the Zellweger patients are able to ω -oxidize but not β -oxidize the bile acid intermediates.

three Zellweger patients contained undetectable amounts of the above chain-shortened metabolites in contrast to the urine from the age-matched control subjects (unpublished observations). In a recent study, 2,3-dinor-thromboxane B₂ was determined in healthy infants, born at full term, during their first week of life. The 2,3-dinor-thromboxane B₂ levels were found to be higher than in adults but declined gradually during the first week of life (31).

In conclusion, the urinary metabolites from intravenously injected prostaglandin $F_{2\alpha}$ were much less oxidized in a patient with the Zellweger syndrome than in two healthy control subjects. The structure of the major urinary metabolite from the Zellweger patient was determined and shown to be 9,11-dihydroxy-15-oxoprost-5-ene-1,20-dioic acid. The major urinary metabolite found in the control subjects (a chain-shortened C_{16} -metabolite) was almost absent in the Zellweger patient. This indicates that peroxisomes are of major importance for the chain shortening of prostaglandins in humans.

Acknowledgments

We would like to thank Dr. Krister Grn, Department of Clinical Chemistry, Karolinska Hospital, for putting radio gas chromatographic facilities at our disposal. The skillful technical assistance of Ms Elisabeth Thorsn is gratefully acknowledged.

This study was supported by a grant to Dr. Alexson and Dr. Diczfalusy from Åke Wibergs stiftelse and by grants to Dr. Alexson from The Bank of Sweden, Tercentenary Foundation and The Swedish Council for Natural Research. One of us (Dr. Björkhem) had a grant from the Swedish Medical Research Council.

References

1. Hamberg, M., and B. Samuelsson. 1971. On the metabolism of prostaglandins E_1 and E_2 in man. *J. Biol. Chem.* 246:6713-6721.
2. Granström, E. 1972. On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. Structure of two metabolites in blood. *Eur. J. Biochem.* 27:462-469.
3. Granström, E., and B. Samuelsson. 1971. On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. *J. Biol. Chem.* 246:5254-5263.
4. Granström, E., and B. Samuelsson. 1971. On the metabolism of prostaglandin $F_{2\alpha}$ in female subjects. II. Structures of six metabolites. *J. Biol. Chem.* 246:7470-7485.
5. Hamberg, M., and B. Samuelsson. 1969. The structure of the major urinary metabolite of prostaglandin E_2 in man. *J. Am. Chem. Soc.* 91:2177-2178.
6. Hamberg, M. 1968. Metabolism of prostaglandins in rat liver mitochondria. *Eur. J. Biochem.* 6:135-146.
7. Johnson, M., P. Davison, and P. W. Ramwell. 1972. Carnitine-dependent β oxidation of prostaglandins. *J. Biol. Chem.* 247:5656-5658.
8. Diczfalusy, U., S. E. H. Alexson, and J. I. Pedersen. 1987. Chain-shortening of prostaglandin $F_{2\alpha}$ by rat liver peroxisomes. *Biochem. Biophys. Res. Commun.* 144:1206-1213.
9. Schepers, L., M. Casteels, J. Vamecq, G. Parmentier, P. P. Van Veldhoven, and G. P. Mannaeys. 1988. β -oxidation of the carboxyl side chain of prostaglandin E_2 in rat liver peroxisomes and mitochondria. *J. Biol. Chem.* 263:2724-2731.
10. Diczfalusy, U., and S. E. H. Alexson. 1988. Peroxisomal chain-shortening of prostaglandin $F_{2\alpha}$. *J. Lipid Res.* 29:1629-1636.
11. Sinikka, H., S. Similä, K. Finni, O. Mäentausta, and O. Jänne. 1980. Cholic acid and chenodeoxycholic acid concentrations in serum during infancy and childhood. *Acta Paediatr. Scand.* 69:659-662.
12. Kase, B. F., J. I. Pedersen, B. Strandvik, and I. Björkhem. 1985. In vivo and in vitro studies on formation of bile acids in patients with the Zellweger syndrome: Evidence that peroxisomes are of importance in the normal biosynthesis of both cholic and chenodeoxycholic acid. *J. Clin. Invest.* 76:2393-2402.
13. Roscher, A., B. Moser, H. Bernheimer, S. Stöckler, I. Mutz, and F. Paltauf. 1984. The cerebrotendofibular (Zellweger) syndrome: an improved method for the biochemical diagnosis and its potential value for prenatal detection. *Pediatr. Res.* 19:930-933.
14. Powell, W. S. 1978. ω -Oxidation of prostaglandins by lung and liver microsomes. *J. Biol. Chem.* 253:6711-6716.

15. Powell, W. S., and S. Solomon. 1977. Formation of 20-hydroxyprostaglandins by lungs of pregnant rabbits. *J. Biol. Chem.* 253:4609-4616.
16. Powell, W. S. 1980. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins*. 20:947-957.
17. Bergström, S., R. Ryhage, B. Samuelsson, and J. Sjövall. 1963. Prostaglandins and related factors. 15. The structures of prostaglandin E₁, F_{1 α} , and F_{1 β} . *J. Biol. Chem.* 238:3555-3564.
18. Goldfischer, S. L., J. Collins, I. Rapin, P. Neumann, W. Neglia, A. J. Spiro, T. Ishii, F. Roels, J. Vamecq, and F. van Hoof. 1986. Pseudo-Zellweger syndrome: deficiencies in several peroxisomal oxidative activities. *J. Pediatr.* 108:25-32.
19. Clayton, P. T., B. D. Lake, M. Hjelm, J. B. P. Stephenson, G. T. N. Besley, R. J. A. Wanders, A. W. Schram, J. M. Tager, R. B. H. Schutgens, and A. M. Lawson. 1988. Bile acid analyses in "Pseudo-Zellweger" syndrome; clues to the defect in peroxisomal beta-oxidation. *J. Inherited Metab. Dis.* 11 (Suppl. 2):165-168.
20. Hamberg, M. 1973. Quantitative studies on prostaglandin synthesis in man. II. Determination of the major urinary metabolite of prostaglandin F_{1 α} and F_{2 α} . *Anal. Biochem.* 55:368-378.
21. Granström, E. 1972. On the metabolism of prostaglandin F_{2 α} in female subjects. Structures of two C₁₄ metabolites. *Eur. J. Biochem.* 25:581-589.
22. Hamberg, M. 1974. Quantitative studies on prostaglandin synthesis in man. III. Excretion of the major urinary metabolite of prostaglandins F_{1 α} and F_{2 α} during pregnancy. *Life Sci.* 14:247-252.
23. Granström, E., H. Kindahl, and M.-L. Swahn. 1982. Profiles of prostaglandin metabolites in the human circulation. Identification of late-appearing, long-lived products. *Biochim. Biophys. Acta.* 713:46-60.
24. Leslie, C. A., and L. Levine. 1973. Evidence for the presence of a prostaglandin E₂ 9-keto reductase in rat organs. *Biochem. Biophys. Res. Commun.* 52:717-724.
25. Sun, F. F. 1974. Metabolism of prostaglandin F_{2 α} in the rat. *Biochim. Biophys. Acta.* 348:249-262.
26. Sun, F. F., and J. E. Stafford. 1974. Metabolism of prostaglandin F_{2 α} in Rhesus monkeys. *Biochim. Biophys. Acta.* 369:95-110.
27. Goldfischer, S., C. L. Moore, A. B. Johnson, A. J. Spiro, M. P. Valsamis, H. K. Wisniewski, R. H. Ritch, W. T. Norton, I. Rapin, and L. M. Gartner. 1973. Peroxisomal and mitochondrial defects in the cerebro-hepato-renal syndrome. *Science (Wash. DC)*. 182:62-64.
28. Kase, B. F., I. Björkhem, P. Hågå, and J. I. Pedersen. 1985. Defective peroxisomal cleavage of the C₂₇-steroid side chain in the cerebro-hepato-renal syndrome of Zellweger. *J. Clin. Invest.* 75:427-435.
29. Bakkeren, J., L. Monnens, J. Trijbels and J. Maas. 1984. Serum very long chain fatty acid pattern in Zellweger syndrome. *Clin. Chim. Acta.* 138:325-331.
30. Hansson, G., and E. Granström. 1977. Metabolism of 15-methyl-prostaglandin F_{2 α} in the cynomolgus monkey and the human female. *Biochem. Med.* 18:420-439.
31. Van Geet, C., J. Arnout, E. Eggermont, and J. Vermeylen. 1990. Urinary thromboxane B₂ and 2,3-dinor-thromboxane B₂ in the neonate born at full-term age. *Eicosanoids.* 3:39-43.