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PARTICIPATION OF THE PEROXISOMAL β-OXIDATION SYSTEM IN THE CHAIN-SHORTENING OF PCA_{16}, A METABOLITE OF THE CYTOSINE ARABINOSIDE PRODRUG, YNKO1, IN RAT LIVER

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Abstract—When PCA_{16}, a metabolite of the cytosine arabinoside prodrug YNKO1, was incubated with isolated rat hepatocytes, time-dependent H_2O_2 generation was found. When the hepatocytes obtained from clofibrate-treated rat liver were used as an enzyme source, PCA_{16}-dependent production of H_2O_2 was increased by around 6-fold. The activity of peroxisomal β-oxidation for PCA_{16} assayed by H_2O_2 generation was 3-fold higher than that for palmitic acid, whereas the activity of mitochondrial β-oxidation for PCA_{16} assayed by ketone body production was much less than that for palmitic acid. A subcellular distribution study revealed that the distribution of the activities of β-oxidation and fatty acyl-CoA oxidase for PCA_{16}-CoA coincided with those of cyanide-insensitive palmitoyl-CoA-dependent β-oxidation and catalase, a marker enzyme of peroxisomes. The profile of the cofactor requirement for β-oxidation of PCA_{16}-CoA in isolated peroxisomes was similar to that for palmitoyl-CoA oxidation, and the reaction was not inhibited by KCN. The formation of CoA derivative prior to β-oxidation reaction was essential. HPLC analysis of metabolites after incubation of PCA_{16}-CoA with isolated peroxisomes demonstrated the production of four metabolites, two of which were identified as PCA_{14} and PCA_{12} by fast atomic bombardment-mass spectrometry. These results indicate that peroxisomal β-oxidation participates in the shortening of the alkyl-side chain of PCA_{16} and plays an important role in the formation of antileukemic cytosine arabinoside from YNKO1.

In 1976, Lazarow and de Duve [1] found that subcellular organelles, peroxisomes, in rat liver had a β-oxidation system different from the mitochondrial system. Since then, many studies have examined the properties of the peroxisomal β-oxidation system to elucidate its physiological role. (1) Peroxisomes can degrade long-chain fatty acids which are hardly susceptible to degradation by the mitochondrial β-oxidation system [2, 3]. (2) Peroxisomes can oxidize some intrinsic acyl-compounds, such as dicarboxylic acids [4], prostaglandin [5] and precursors of bile acids [6]. Furthermore, we have shown that xenobiotic acyl-compounds are chain-shortened exclusively by the peroxisomal β-oxidation system [7–9]. Thus, in contrast to the mitochondrial β-oxidation system, which mainly contributes to energy production in the biological system, peroxisomal β-oxidation may function as a catabolic system which does not relate directly to energy production.

There are many drugs which are metabolized by β-oxidation. These drugs have acyl or alkyl groups as side chains. Thus, it may be that the peroxisomal β-oxidation system may take part in the metabolism of such drugs.

YNKO1 is a prodrug of cytosine arabinoside (Ara-C) [10] derived by modification of its sugar moiety with stearylphosphate [11]. YNKO1 is in the developmental stage as an antileukemic drug (Chart 1). Clinically, Ara-C is easily inactivated in the body by deamination [12]. YNKO1 has been developed as a prodrug to inhibit such inactivation and, as a consequence, to result in a longer delivery time of Ara-C. When YNKO1 was administered to rats, PCA_4 and Ara-C were found as the main metabolites in the liver, suggesting that YNKO1 could be chain-shortened by ω-oxidation and subsequent β-oxidation [13].

In the present study we have used PCA_{16}, a metabolic intermediate of this pathway, to examine whether peroxisomal β-oxidation participates in the chain-shortening of YNKO1.

MATERIALS AND METHODS

PCA_n were prepared in the Research Laboratories of Yamasa Shoyu Co. Ltd (Choshi, Japan). PCA_{16}-CoA was prepared by the method of Kawaguchi *et al.* [14] and purified by HPLC using μBondapak C18 (7.8 × 30 cm) and CH_3OH, 0.1 M sodium phosphate buffer (pH 7.0) (66:34) as a solvent. NAD, CoA, ATP, palmitoyl-CoA and fatty acid-free bovine serum albumin (BSA) were purchased from the Sigma Chemical Co (St Louis, MO). Other chemicals, all of reagent grade, were obtained from commercial sources.

Animals and treatment. Male Wistar rats, weighing about 250 g, were used. Clofibrate treatment was performed by feeding regular chow diet (Clea Japan

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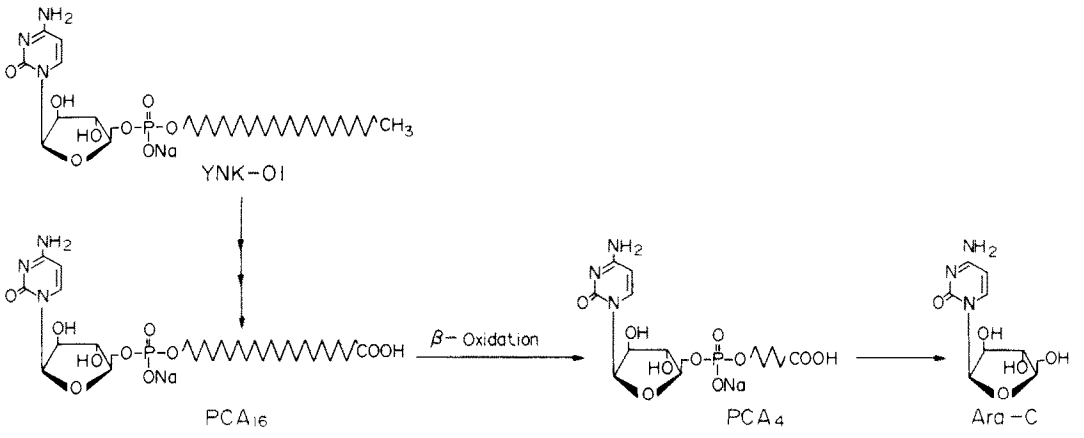

Chart 1. Apparent metabolic pathway of YNKO1.

Inc., Tokyo, Japan) containing 0.25% clofibrate for 2 weeks. Some rats were killed by decapitation, and the livers were removed and homogenized in 0.25 M sucrose. The homogenates were used for determination of enzyme activities. Other rats were used for the preparation of isolated hepatocytes.

Subcellular fractionation of the liver. The liver homogenate was fractionated by the method of de Duve *et al.* [15]. The light mitochondrial fraction (LM fraction) was further centrifuged in a discontinuous sucrose density gradient [16].

Preparation of mitochondria. Mitochondria was prepared by the method of Hoppel *et al.* [17]. The liver homogenate (10%, w/v) prepared from untreated rats in 0.3 M mannitol–1 mM EDTA–10 mM HEPES* (pH 7.4) was centrifuged at 400 *g* for 10 min, and the supernatant fraction was centrifuged at 7000 *g* for 10 min with washing once at each centrifugation.

Experiments with isolated hepatocytes. Hepatocytes were prepared from control and clofibrate-treated rats by the collagenase-perfusion method of Moldeus *et al.* [18]. Cell preparations, showing more than 90% cell viability in terms of lactate dehydrogenase-latency and the trypan blue exclusion test, were used. PCA_{16}-dependent H_2O_2 generation based on peroxisomal β-oxidation was determined by peroxidatic generation of formaldehyde from methanol [19]. The mixture (2 mL), containing 2×10^6 cells/mL, 1 mM PCA_{16} (acid form) or palmitate, 20 mg/mL BSA, 50 mM methanol, 10 mM semicarbazide, 1 mM 4-methylpyrazole and Krebs–Henseleit buffer (pH 7.4), was incubated at 37° for various time intervals. The reaction was then stopped by addition of 55% trichloroacetic acid (0.2 mL) and the mixture centrifuged. The formaldehyde content of the supernatant fraction was measured by the method of Nash [20]. Ketone body-formation based on the mitochondrial β-oxidation of PCA_{16} was determined by the method of Leighton *et al.* [21]. In brief, isolated hepatocytes were preincubated with 1 mM L-carnitine at 37° for 20 min. The reaction mixture (2 mL), containing 0.5×10^6 cells/mL, 1 mM PCA_{16} (acid form) or palmitate, 0.5 mM L-carnitine, 20 mg/mL BSA and Krebs–Henseleit buffer (pH 7.4), was incubated at 37° for 20 min, deproteinized with trichloroacetic acid and centrifuged. The ketone body content (acetoacetic acid and 3-hydroxybutyric acid) in the supernatant fraction was determined by the method of Williamson *et al.* [22].

Analysis of metabolites derived from PCA_{16} by peroxisomal β-oxidation. Analysis of the metabolites of PCA_{16} was performed as follows: The reaction mixture (10 mL), containing 0.05 mM PCA_{16}-CoA, 0.2 mM NAD, 0.05 mM CoA, 1 mM KCN, 6 mM dithiothreitol, 0.01% Triton X-100, 0.15 mg/mL BSA, 30 mM potassium phosphate buffer (pH 7.4) and 1 mL of enzyme preparation, was incubated at 37° for 120 min. Then 1 mL of 5 M KOH was added, and the mixture was incubated at 60° for 20 min and neutralized with diluted HCl. The solution was applied on a SEP-PAK C_{18} cartridge column and eluted with CH_3OH–H_2O (80:20). For determination of long-chain metabolites, and aliquot of the eluate was concentrated by evaporation at 40°, neutralized with NaOH, lyophilized, solubilized in 500 μL of 0.1 M NaOH, and applied to HPLC. The residual part was applied on a Dowex 1-X2 column (1×5 cm) for the determination of short-chain metabolites and eluted with 1 M HCl, and the eluate was processed as described above and then subjected to HPLC. The conditions of HPLC analysis are described in the figure legends. Identification of metabolites was performed by use of a VG Micromass ZAB-HF mass spectrometer (VG Analytical Co. Ltd).

Enzyme analysis. Cyanide-insensitive β-oxidation activity (FAOS) for PCA_{16}-CoA or palmitoyl-CoA was determined spectrophotometrically by following CoA derivative-dependent NAD reduction by the method of Lazarow and de Duve [1]. Acyl-CoA oxidase activity (FAO) of PCA_{16}-CoA was determined spectrophotometrically by following PCA_{16}-CoA-dependent H_2O_2 generation by the method of Osumi *et al.* [23]. One unit of these activities was

* Abbreviations: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; and EGTA, ethyleneglycolbis(aminoethylether)tetra-acetate.

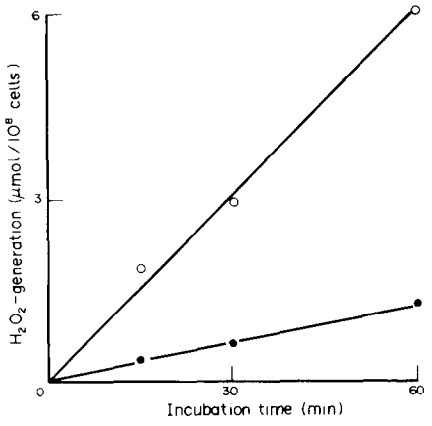

Fig. 1. PCA_{16}-dependent H_2O_2 generation in isolated hepatocytes from control (●) and clofibrate-treated (○) rats.

Table 1. Effects of clofibrate on peroxisomal β-oxidation activities for PCA_{16} in rat liver

	Enzyme activity (units/g liver)	
	NAD reduction	H_2O_2 generation
PCA_{16}		
Untreated	46 ± 3	73 ± 7
Clofibrate	410 ± 29	710 ± 37
Palmitate		
Untreated	528 ± 49	663 ± 71
Clofibrate	4421 ± 176	7303 ± 394

With use of liver homogenates, and Co-A derivatives of PCA_{16} and palmitic acid as substrate, peroxisomal β-oxidation activities were followed by cyanide-insensitive acyl-CoA-dependent NAD reduction or H_2O_2 generation as described in Materials and Methods. Results are the means ± SD of three experiments. Units: nmol/min.

defined as the amount of enzyme which produced 1 nmol of NADH or H_2O_2/min under the assay conditions. Acyl-CoA dehydrogenase (FADH) activity was determined by the method of Hryb and Hogg [24]. One unit of activity was defined as the amount of enzyme which reduced 1 nmol 2,4-dichlorophenol indophenol/min under the assay conditions. Acyl-CoA synthetase activity was determined by the method of Tanaka *et al.* [25] with the use of HPLC. One unit of activity was defined as the amount of enzyme which produced 1 nmol acyl-CoA/min under the assay conditions. Carnitine acyltransferase (CAcyl-T) activity was determined by the method of Markwell *et al.* [26]. One unit of activity was defined as the amount of enzyme which produced 1 nmol acyl-CoA/min under the assay conditions. Carnitine acyltransferase (CAcyl-T) activity was determined by the method of Markwell *et al.* [26]. One unit of activity was defined as the amount of enzyme which released 1 nmol CoA-SH/min. When isolated mitochondria were used for the assay of mitochondrial β-oxidation activity, substrate-dependent O_2 consumption was followed by polarography. The assay medium (1.6 mL) contained 75 μM substrate (CoA-ester form), 3 mM ADP, 1 mM ATP, 0.5 mM CoA, 2 mM $MgCl_2$, 2 mM dithiothreitol, 2 mM L-carnitine, 2 mM potassium phosphate, 10 mM Tris–malonate, 2.4 mg fatty acid-free bovine serum albumin, 130 mM KCl, 10 mM HEPES (pH 7.4), 0.1 mM EGTA and 80 μL isolated mitochondria from the untreated rat liver (average 1.5 mg protein). The measurement was carried out in the presence of 1 μg/mL antimycin A as a control. The activities of catalase and glutamate dehydrogenase (GDH) were determined by the methods of Luck [27] and Beaufay *et al.* [28] respectively. Protein content was determined by the method of Lowry *et al.* [29] using BSA as a standard.

RESULTS

β-Oxidation of PCA_{16} in isolated rat hepatocytes. When PCA_{16} was incubated with rat hepatocytes obtained from untreated controls, PCA_{16}-dependent H_2O_2 generation based on peroxisomal β-oxidation was found to be linear for 60 min. When using the hepatocytes obtained from clofibrate-treated rat liver, the rate of H_2O_2 generation was enhanced by 6-fold, as shown in Fig. 1. The activities of peroxisomal and mitochondrial β-oxidation for PCA_{16} and palmitic acid in isolated hepatocytes are shown in Fig. 2. These activities were determined on the basis of H_2O_2 generation and ketone body formation. The activity of peroxisomal β-oxidation for PCA_{16} was about 3-fold higher than that for palmitic acid, whereas that of mitochondrial β-oxidation for PCA_{16} was about one twentieth that for palmitic acid.

β-Oxidation of PCA_{16} by rat liver peroxisomes. The activities of FAOS and FAO in the homogenates of untreated and clofibrate-treated rat liver, using PCA_{16}-CoA as substrate, were determined (Table 1). Although the activities of FAOS and FAO in the untreated homogenate for PCA_{16}-CoA were about one-tenth of those for palmitoyl-CoA, the activities for PCA_{16}-CoA of clofibrate-treated rat liver were enhanced about 10-fold in parallel to the changes in the case when palmitoyl-CoA was used as a substrate. The subcellular distribution pattern of the activity of β-oxidation for PCA_{16}-CoA is shown in Fig. 3. Catalase and glutamate dehydrogenase were determined as marker enzymes for the peroxisomes and mitochondria. The distribution pattern of FAOS and FAO activities for PCA_{16}-CoA were consistent with those of catalase and FAOS activity for palmitoyl-CoA, indicating that the FAOS and FAO activities for PCA_{16}-CoA were localized in the peroxisomal fraction. The cofactor requirement of the PCA_{16}-oxidation reaction by the peroxisomal fraction obtained from sucrose density gradient centrifugation of the light mitochondrial fraction of rat liver was examined (Table 2). When PCA_{16}-free acid was used as a substrate instead of PCA_{16}-CoA, the oxidation activity was decreased markedly. It was, however, restored to about 70% of that seen with PCA_{16}-CoA thioester by the addition of CoA, ATP and $MgCl_2$. Furthermore, after preincubation before starting the reaction by the addition of NAD, the decreased activity was restored to about 90% of that of the complete system. In this preincubation,

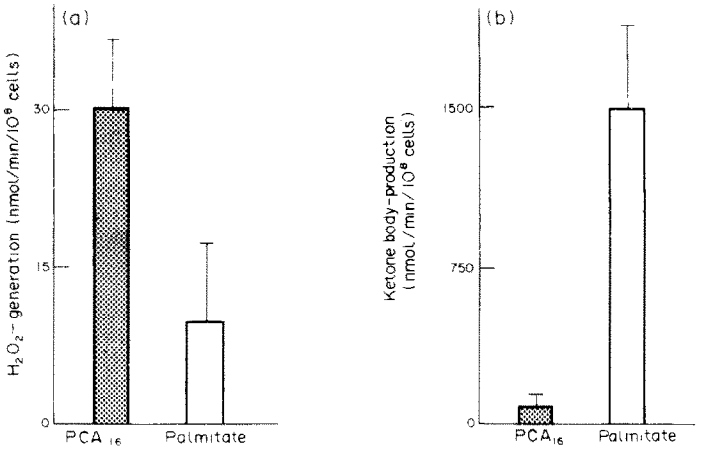

Fig. 2. Activities of peroxisomal amd mitochondrial β-oxidation in isolated rat hepatocytes, which were measured by following H_2O_2 generation (a) or ketone body production (b) respectively. Results are the means ± SD of three rats.

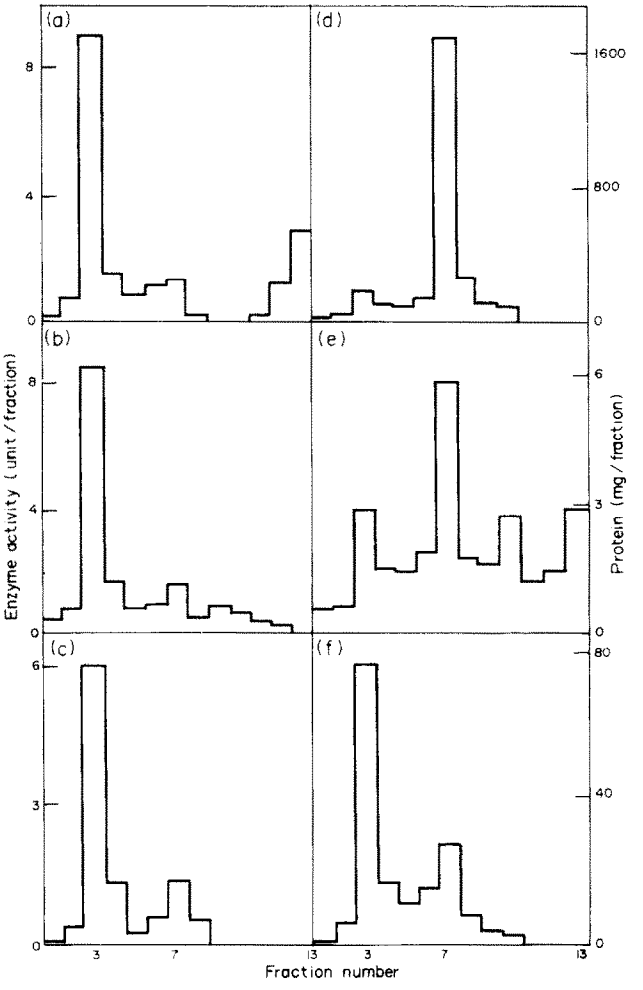

Fig. 3. Sucrose density gradient centrifugation of light mitochondrial fraction. Key: (a) catalase; (b) FAO (for PCA_{16}-CoA); (c) FAOS (for PCA_{16}-CoA); (d) glutamate dehydrogenase; (e) protein; and (f) FAOS (for palmitoyl-CoA).

Table 2. Cofactor requirement for PCA_{16}-dependent NAD reduction by the peroxisomal fraction obtained from sucrose density gradient centrifugation of the light mitochondrial fraction of untreated rat liver

Condition	Specific activity (units/mg protein)	
	Substrate	
	PCA_{16}-CoA	Palmitoyl-CoA
(1) Complete system*	4.6	29.8
(2) Free acid form as substrate†	ND‡	ND
(3) (2) + CoA, ATP and $MgCl_2$	3.2	16.7
(4) (3) + preincubation	4.1	30.1
(5) (1) or (4) − Enzyme source	ND	ND
(6) (1) or (4) − Substrates	ND	ND
(7) (1) or (4) − NAD	ND	ND
(8) (1) or (4) − CoA	3.3 or ND	23.3 or 2.7
(9) (1) or (4) − KCN	4.2 or 4.3	27.1 or 28.3

The basic reaction mixture (1.0 mL) contained 0.05 mM CoA, 0.2 mM NAD 1 mM KCN, 6 mM DTT, 0.01% Triton X-100, 0.15 mg BSA, 30 mM potassium phosphate (pH 7.4) and the peroxisomal fraction (35.6 μg protein). The following cofactors were added: 5 mM ATP, 5 mM $MgCl_2$ and 0.6 mM CoA. Preincubation in (4) was carried out at 37° for 20 min before starting the reaction by the addition of NAD.

* 0.05 mM PCA_{16}-CoA and palmitoyl-CoA were used as substrates.

† 0.2 mM PCA_{16} acid form and 0.1 mM palmitic acid were used as substrates.

‡ ND, not detectable.

Table 3. Acyl-CoA synthetase activity for PCA_{16} in untreated rat liver homogenates

Substrate	Activity (units/g liver)
PCA_{16} (acid form)	5107 ± 618
Palmitic acid	57,000 ± 1253

Results are the means ± SD of three experiments. Units: nmol/min.

PCA_{16}-CoA might be formed by intrinsic acyl-CoA synthetase from the free acid form. Table 3 shows the activity of acyl-CoA synthetase in untreated rat liver homogenates for PCA_{16} and palmitic acid. Although the activity of acyl-CoA synthetase for PCA_{16} was 5017 units/g liver and about one-tenth of that for palmitic acid, the activity was sufficiently high for the supply of PCA_{16}-CoA into the peroxisomal β-oxidation system. The HPLC chromatograms of the extracts from the reaction mixture after PCA_{16}-CoA was incubated with the peroxisomal fraction are shown in Fig. 4. After 120 min of incubation, four peaks were found. These were recognized as metabolites on the basis of the cofactor requirement and time course of this reaction (data not shown). On comparison with the retention time of standards and analysis by fast atomic bombardment-mass spectrometry (Fab-MS), metabolites 3 and 4, which have molecular weights of 521 and 549 on the basis of the presence of MH^+ and MNa, were identified as PCA_{12} and PCA_{14}. Although identification of metabolites 1 and 2 by Fab–MS was not achieved, these seem to be PCA_6 and PCA_{10} on the basis of their retention times. An extent of the contribution of mitochondrial and peroxisomal β-oxidation to the shortening of the side chain of PCA_{16} was estimated (Table 4). The capacities in the liver of β-oxidation of PCA_{16}-CoA by peroxisomes and mitochondria were calculated based on the activities followed by NAD reduction as peroxisomal and by O_2 consumption as mitochondrial using the peroxisomal fraction obtained from sucrose density gradient centrifugation and isolated mitochondria, respectively, and also on the recoveries of marker enzymes in those fractions from the starting liver homogenate. Although the peroxisomal β-oxidation activity for palmitoyl-CoA in the liver was one-fourth of that of mitochondrial activity and the peroxisomal activity for PCA_{16}-CoA was one-tenth of that for palmitoyl-CoA, no significant activity of β-oxidation for PCA_{16}-CoA in mitochondria was detected. Furthermore, the activities of FADH and CAcyl-T for PCA_{16}-CoA, which were involved in mitochondrial fatty acid metabolism, were extremely low, compared with those for palmitoyl-CoA as a substrate. CAcyl-T activity was not detected (Table 5).

DISCUSSION

Some drugs have alkyl-, alcohol- or acyl-side chains in their structures and, when administered, chain-shortened derivatives have been found as metabolites. Upon administration of YNKO1, a cytosine arabinoside prodrug, to rats, PCA_4 and Ara-C were found as major metabolites [13] in livers, suggesting that the ω- and β-oxidation systems participate in the chain-shortening of YNKO1. We have examined the metabolism of YNKO1 in rat liver by using PCA_{16}, an expected intermediary metabolite. When PCA_{16} was incubated with isolated rat hepatocytes, PCA_{16}-dependent H_2O_2 generation was

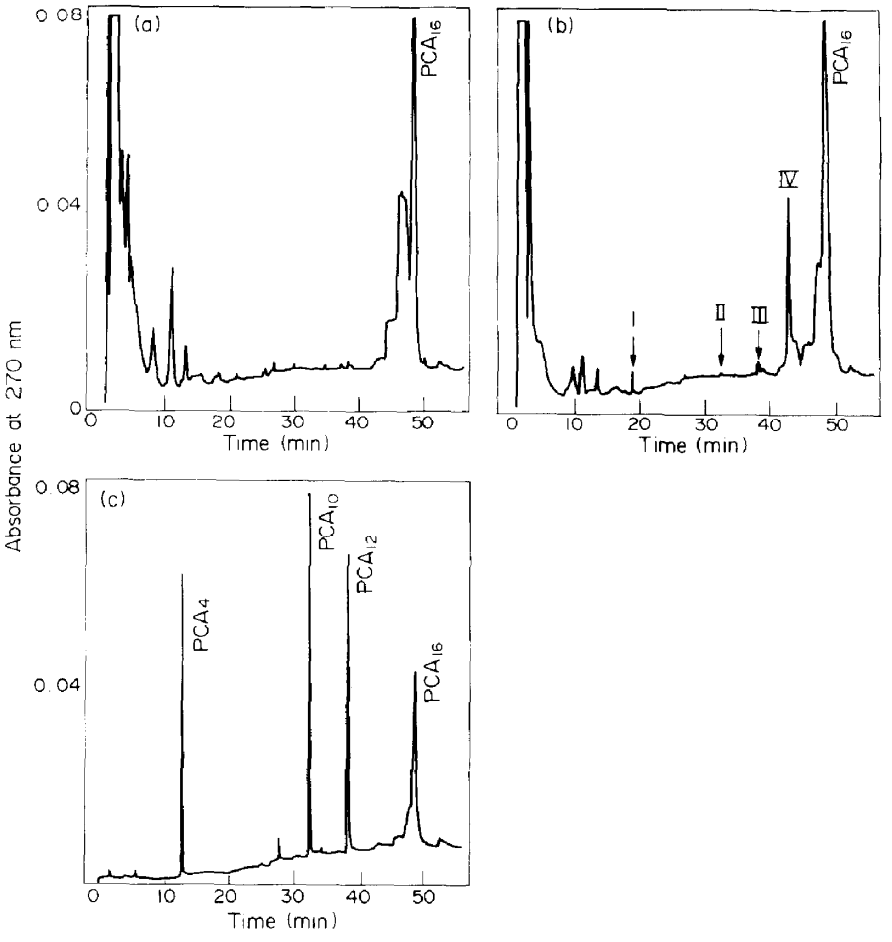

Fig. 4. HPLC chromatograms of the products obtained from incubations of PCA_{16} with peroxisomes. Key: (a) non-incubated reaction mixture; (b) 120-min incubated reaction mixture; and (c) standard; (I) metabolite 1; (II) metabolite 2; (III) metabolite 3, and (IV) metabolite 4. Chromatographic conditions: column, NOVA-PAK C18 (3.9 mm × 15 cm); solvent CH_3OH–0.65% CH_3COOH (0–5 min, 0.65% CH_3COOH 70% CH_3OH at 2%/min); flow rate, 1 mL/min; detector, UV monitor set at 270 nm.

Table 4. Comparison between peroxisomal and mitochondrial β-oxidation activities for PCA_{16}- and palmitoyl-CoA

Substrate	Activity (units/g liver)	
	Peroxisomes	Mitochondria
PCA_{16}-CoA	54 ± 11	ND*
Palmitoyl-CoA	535 ± 78	2107 ± 103

Using peroxisomal and mitochondrial fractions obtained from the sucrose density gradient centrifugation of the light mitochondrial fraction of untreated rat liver, the capacities of β-oxidation in peroxisomes and mitochondria were examined by following NAD reduction (1) and O_2 consumption, respectively, as described in Materials and Methods. Results are the means ± SD of four experiments. Units: nmol/min.

* ND, not detectable.

Table 5. Mitochondrial activities of β-oxidation-related enzymes for PCA_{16}- and palmitoyl-CoA

Substrate	Activity (units/g liver)	
	FADH	CAcyl-T
PCA_{16}-CoA	59 ± 22	ND*
Palmitoyl-CoA	1814 ± 479	2207 ± 365

The activities of mitochondrial FADH and CAcyl-T in untreated rat liver were determined using the mitochondrial fraction obtained from sucrose density gradient centrifugation as an enzyme source. Results are the means ± SD of three experiments. Units: nmol/min.

* ND, not detectable.

found, and the rate was enhanced markedly in the hepatocytes from clofibrate-treated rats. These results suggest that PCA_{16} incorporated into hepatocytes is chain-shortened by peroxisomal β-oxidation, because unlike mitochondria, the key enzyme of the peroxisomal β-oxidation system is fatty acyl-CoA oxidase, and the activity is increased markedly by clofibrate, a peroxisome proliferator [1–3]. Hepatocytes, however, have two β-oxidation systems, the mitochondrial and the peroxisomal systems. To clarify the contribution of the peroxisomal β-oxidation system to the metabolism of PCA_{16}, we compared the peroxisomal β-oxidation activity for PCA_{16} with that of mitochondria. The peroxisomal activity was calculated as 30.1 nmol/min/10^6 cells while that of the mitochondria was not significant on the basis of H_2O_2 generation and ketone body formation respectively. Considering that the variation of the activity of mitochondria was marked, and the apparent peroxisomal activity found in this experiment would be about 50% of the true activity because of degradation of generated H_2O_2 by catalatic activity of catalase, the direct comparison of the activities of the two β-oxidation systems for PCA_{16} may not be adequate. However, these results show that PCA_{16} can be oxidized by peroxisomes more easily than by mitochondria in liver cells. This was also supported by the experiments with isolated peroxisome fractions. In rat liver homogenate the activities of FAOS and FAO for PCA_{16}-CoA could be detected, and the activities were one-tenth of those for palmitoyl-CoA. These activities in the liver were enhanced markedly after *in vitro* treatment with a peroxisome proliferator, clofibrate. The sucrose density gradient centrifugation experiment showed that these activities for PCA_{16}-CoA were localized in the peroxisomal fraction. The cofactor requirement of the peroxisomal β-oxidation system for PCA_{16}-CoA was similar to that for palmitoyl-CoA oxidation, indicating that PCA_{16}-CoA may be oxidized in the same pathway as palmitoyl-CoA. Furthermore, it was essential for peroxisomal β-oxidation of PCA that PCA_{16} was activated to CoA derivatives. Although the activity of acyl-CoA synthetase for PCA_{16} in the liver was 5017 units/g liver and about one-tenth of that for palmitic acid, the activity was sufficient for supplying PCA_{16}-CoA to the peroxisomal β-oxidation pathway. Analysis of the metabolites by HPLC showed that the production of PCA_{12} and PCA_{14} occurred in the peroxisomes, and no production of metabolites having a carbon side chain with odd-numbers was observed, indicating that PCA_{16} may be degraded by two carbon units. In the experiment with isolated mitochondria, no significant β-oxidation activity for PCA_{16}-CoA was observed. Furthermore, the activities of FADH and CAcyl-T, which are the enzymes in the mitochondrial fatty acid oxidation systems, for PCA_{16}-CoA were extremely low, and no activity of CAcyl-T was detectable. This suggests that if any activity exists in the mitochondrial β-oxidation pathway for PCA_{16}-CoA, the step of the incorporation of PCA_{16}-CoA into mitochondria may be the rate-limiting step. From these results it is concluded that the process of chain-shortening of PCA_{16} in the liver is performed exclusively by the peroxisomal β-oxidation system. Therefore, peroxisomes play an important role in the metabolic activation of a prodrug, YNKO1, resulting in release of the antileukemic agent, Ara-C. However, for chain shortening of YNKO1 by β-oxidation, hydroxylation at the alkyl-end of the side-chain and subsequent oxidation to the carboxylic acid form are essential as a first step. The mechanism of this first step remains to be clarified. It is widely known that the ω- and (ω-1) oxidation of fatty acids is catalyzed by microsomal cytochrome P-450 species [30–32]. The hepatic activity was increased by peroxisome proliferators, such as clofibrate and di-(2-ethylhexyl)-phthalate (DEHP) [33, 34]. Tamburini *et al.* [35] showed that the increase in the activity of fatty acid hydroxylation after peroxisome proliferator treatment is associated with the increase in the hepatic content of a specific cytochrome P-450 species, cytochrome P-452. However, subcellular distribution showed that the hydroxylation activity for fatty acids was localized exclusively in microsomes, not in peroxisomes (data not shown). Thus, YNKO1 incorporated into hepatic cells may be initially hydroxylated by cytochrome P-450 species in microsomes, further oxidized to the acid form, and then incorporated into peroxisomes as free acid and/or an acyl-CoA.

Recently, many prodrugs, which were derivatized by chemical modification of parent compounds, have been developed either to allow their toxicity to be decreased and/or pharmacological activities to be sustained. In the process of the design of such drugs, the metabolism of the drugs by peroxisomal β-oxidation may be meaningful for evaluation of the pharmacological activity and toxicity of the drugs.

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