Experimental Cell Research 170 (1987) 147–152

Peroxisomes and Peroxisomal Functions in Muscle

Studies with Muscle Cells from Controls and a Patient with the Cerebro-Hepato-Renal (Zellweger) Syndrome

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In the present study we investigated peroxisomal functions in cultured human muscle cells from control subjects and from a patient with the Zellweger syndrome, a genetic disease characterized by the absence of morphologically distinguishable peroxisomes in liver and kidney. In homogenates of cultured muscle cells from control subjects, catalase is contained within subcellular particles, acyl-CoA : dihydroxyacetonephosphate acyltransferase activity is present and palmitoyl-CoA can be oxidized by a peroxisomal β-oxidative pathway; these findings are indicative of the presence of peroxisomes in the cells. In homogenates of cultured muscle cells from the patient with the Zellweger syndrome, acyl-CoA : dihydroxyacetonephosphate acyltransferase activity was deficient, peroxisomal β-oxidation of palmitoyl-CoA was impaired and catalase was not particle-bound. These findings indicate that functional peroxisomes are absent in muscle from patients with the Zellweger syndrome. We conclude that cultured human muscle cells can be used as a model system to study peroxisomal functions in muscle and the consequences for this tissue of a generalized dysfunction of peroxisomes.

Peroxisomes carry out a diverse set of metabolic functions and are now recognized to be nearly ubiquitous in eukaryotic cells [1]. After the first description of these particles in the cytoplasm of mouse proximal kidney tubules and rat hepatocytes [2, 3], peroxisomes were first characterized as biochemically distinct organelles by de Duve and co-workers (for review, see [4]). Although peroxisomes were long believed to play only a modest role in mammalian metabolism, it is now clear that they are involved in a number of metabolic processes including ether-phospholipid biosynthesis [5, 6], very long chain fatty acid oxidation [7] and bile acid synthesis [8]. The recognition in recent years of a new group of inherited diseases in man in which one or more peroxisomal functions are impaired (for review, see [9–11]), stresses the importance of peroxisomes.

At present it is possible to establish cultures of several different cell types from tissue samples obtained by biopsy, and cryopreservation methods offer the

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0014-4827/87 $03.00

possibility of studying the cells for long periods of time. Muscle explants, for instance, can readily be established in culture and the proliferating cells show a remarkable degree of differentiation [12]. Cultured muscle cells have now become a useful source of material for the identification of inborn errors of metabolism [12].

The aim of the present study was to investigate whether cultured human muscle cells can be used for the study of peroxisomes and peroxisomal functions in genetic diseases like the cerebro-hepato-renal (Zellweger) syndrome in which peroxisomes are known to be absent [13] and peroxisomal functions are impaired [9–11]. The results are described in this paper.

MATERIALS AND METHODS

Cell Culture Procedures

Human muscle cells were cultured by a modification [14] of the method described by Yasin et al. [15]. Muscle samples (about 0.1 g wet wt) were taken from the vastus lateralis muscle by biopsy. The muscle tissue was dissociated by incubation with trypsin and collagenase exactly as described [14]. The mononucleated cells were cultured at 37°C in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal calf serum (FCS), 2% detoxified chick embryo extract and antibiotics (see [14]).

Measurement of the Subcellular Localization of Enzymes in Cultured Human Muscle Cells

Intact cultured muscle cells harvested in a medium containing 300 mM sucrose plus 10 mM HEPES (pH 7.4) were incubated in isotonic media containing different concentrations of digitonin and the free, i.e. non-latent, activity of lactate dehydrogenase and catalase was measured as follows as described before [16]. Lactate dehydrogenase was assayed by recording the decrease in absorbance at 340 nm in a medium (2 ml) containing cultured muscle cells (about 10 μg protein/ml), 300 mM sucrose, 5 mM HEPES, 0.2 mM NADH and 0.5 mM pyruvate; the final pH was 7.4.

Activity Measurements

Catalase [16], acyl-CoA : dihydroxyacetone phosphate acyltransferase [17] and peroxisomal palmitoyl-CoA β-oxidation [18] were measured as described.

Patient

The patient used in the present study, the child of two healthy unrelated parents, showed the clinical and biochemical abnormalities characteristic for the cerebro-hepato-renal (Zellweger) syndrome (see refs [9–11]). Biochemical abnormalities in this patient included the accumulation in serum of abnormal bile acids (di- and trihydroxycoprostanoic acid), very long chain fatty acids, pipecolic acid and phytanic acid and a deficiency in cultured skin fibroblasts of the peroxisomal enzyme acyl-CoA : dihydroxyacetone phosphate acyltransferase. Morphologically distinct peroxisomes were absent in a liver biopsy specifically prepared for the detection of peroxisomes according to the method described by Herzog & Fahimi [19].

Materials

Nucleotides and enzymes were purchased from Boehringer (Mannheim, FRG) or Sigma (St Louis, Mo.). Radiochemicals were from The Radiochemical Centre (Amersham, Bucks, England), all other reagents were of analytical grade.

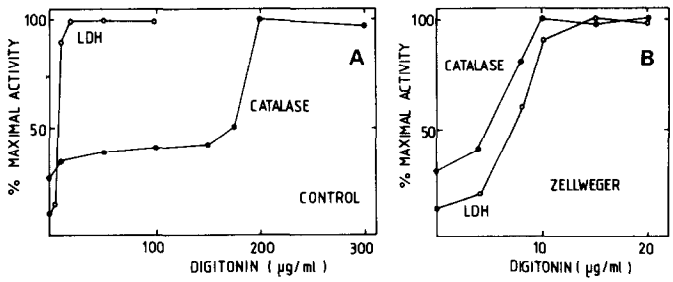

Fig. 1. Measurement of particle-bound catalase in cultured human muscle cells (myoblasts) from a control subject and a patient with the Zellweger syndrome. Myoblasts from a control subject (*A*) and a patient with the Zellweger syndrome (*B*) were incubated in isotonic sucrose containing the amounts of digitonin indicated and the free activity of lactate dehydrogenase and catalase was measured as described before [16]. Open symbols, lactate dehydrogenase; closed symbols, catalase.

RESULTS

Under the culture conditions used, cell division resulted in a doubling of the cell number within about 30 h until the cell density reached a value of about 2×10^4 cells/cm^2. At this density the muscle cells started to fuse and an increasing number of myotubes was found. The results of table 1 indicate that catalase is present in large amounts both in cultured human myoblasts and myotubes, the activity being about 5 and 20% of that found in human liver and cultured skin fibroblasts, respectively [16].

We have recently described a method for determining the subcellular localization of enzymes in cultured skin fibroblasts [16]. The method is based upon the use of digitonin to selectively permeabilize the plasma membrane and the different intracellular membranes and upon the lack of activity of an enzyme due to the presence of an impermeable membrane preventing free accessibility of the substrate to the enzyme. We have now used this method to study the intracellular localization of catalase in cultured myoblasts and myotubes. Fig. 1 shows the results of a representative experiment with cultured myoblasts.

The activities of lactate dehydrogenase and catalase were measured by incubating cultured myoblasts in a buffered isotonic sucrose medium containing different concentrations of digitonin. The results indicate that small amounts of digitonin were required to release the latency of lactate dehydrogenase in these cells. In contrast to the situation with lactate dehydrogenase, the release of the latency of catalase showed a biphasic pattern: at low digitonin concentrations, about 40% of the total catalase activity was unmasked, whereas the bulk of the catalase activity was elicited at much higher digitonin concentrations. This result closely reflects the situation in cultured skin fibroblasts [16]. It can be concluded from these results that in cultured muscle cells, most of the catalase is contained within subcellular particles.

We also investigated whether the peroxisomal membrane-bound enzyme acyl-CoA : dihydroxyacetone phosphate acyltransferase is present in cultured muscle

Table 1. *Activity and subcellular localization of catalase and the activity of acyl-CoA : dihydroxyacetone phosphate acyltransferase and peroxisomal palmitoyl-CoA β-oxidation in cultured human myoblasts and myotubes established from biopsies obtained from control subjects*

Parameter measured	Myoblasts		Myotubes	
Catalase activity (μmol/min · mg protein)	2.18±0.42	(5)	1.9±0.3	(3)
Particle-bound catalase (% of total)	65±9	(5)	58±9	(3)
Acyl-CoA : dihydroxyacetone phosphate acyltransferase (nmol/2 h · mg protein)	8.8±2.5	(5)	10.1±2.1	(3)
Peroxisomal palmitoyl-CoA β-oxidation activity (nmol/min · mg protein)	0.30±0.05	(5)	ND	

Enzyme activities and the percentage of particle-bound catalase were determined as described in Materials and Methods, with the number of different cell preparations within parentheses. ND, Not determined.

cells. As shown by De Clerq et al. [20] this enzyme activity when assayed at pH 5.5 in the presence of surplus glycerol 3-phosphate, represents an ideal marker enzyme for peroxisomes. Table 1 shows that cultured muscle cells do, indeed, contain this enzyme activity. The values obtained are similar to those found in cultured human skin fibroblasts (compare [16, 17]).

Mammalian peroxisomes contain a fatty acid β-oxidation system distinct from the mitochondrial system (for review, see [21]). Peroxisomal β-oxidation activity has been demonstrated in skeletal muscle from different species (see e.g. [22–26]). Using an assay system based upon the peroxisomal acyl-CoA oxidase-

Table 2. *Activity and subcellular localization of catalase and the activity of acyl-CoA : dihydroxyacetone phosphate acyltransferase and peroxisomal palmitoyl-CoA β-oxidation in cultured human myoblasts and myotubes from a patient with the Zellweger syndrome*

Parameter measured	Myoblasts		Myotubes
	Expt 1	Expt 2	
Catalase activity (μmol O_2/min · mg protein)	3.5	3.8	2.8
Particle-bound catalase (% of total)	≤5	≤5	≤5
Acyl-CoA : dihydroxyacetone phosphate acyltransferase activity (nmol/2 h · mg protein)	1.07	N.D.	N.D.
Peroxisomal palmitoyl-CoA β-oxidation activity (nmol/min · mg protein)	N.D.	0.10	N.D.

For experimental details, see Materials and Methods. Results are means of duplicate or triplicate measurements. ND, not determined.

catalysed production of acetyl units from palmitoyl-CoA (see [18]), we found (table 3) that cultured human muscle cells contain an appreciable peroxisomal β-oxidation capacity.

In 1973 Goldfischer et al. [3] described the absence of morphologically distinct peroxisomes in hepatocytes and renal tubule cells of Zellweger patients. In order to investigate whether the deficiency of peroxisomes was also reflected in muscle of Zellweger patients we investigated the subcellular localization of catalase, the activity of acyl-CoA : dihydroxyacetone phosphate acyltransferase and the peroxisomal β-oxidation of palmitoyl-CoA in cultured muscle cells from a Zellweger patient. The results summarized in table 2 indicate that catalase is not contained within subcellular organelles in the patient's cells but is present in the cytosol. Furthermore, both acyl-CoA : dihydroxyacetone phosphate acyltransferase and the peroxisomal palmitoyl-CoA oxidation capacity were strongly deficient in these cells.

DISCUSSION

The results described in this paper indicate that in cultured human muscle cells the bulk of the catalase (about 60%) is contained within a subcellular organelle, thus providing evidence for the existence of peroxisomes in these cells. Further support for the presence of peroxisomes is provided by the peroxisomal palmitoyl-CoA β-oxidation activity and acyl-CoA : dihydroxyacetone phosphate acyltransferase activity found in these cells (table 1). The results of table 2 indicate that peroxisomes are severely deficient in cultured muscle cells from a Zellweger patient. The latter finding opens up several interesting possibilities.

First, the consequences of a generalized loss of peroxisomal functions on growth, differentiation and functioning of muscle can be studied in cultured muscle cells from Zellweger patients; it should be pointed out that hypotonia and muscle weakness are characteristic abnormalities in Zellweger syndrome [9–11]. Furthermore, since mitochondrial abnormalities have been described in patients with the Zellweger syndrome (see [9–11]), the effect of the inability of these mutant muscle cells to synthesize a major class of phospholipids, the plasmalogens, on for instance the oxidative functions of muscle mitochondria can be studied, which might provide further information on the relationship between peroxisomal and mitochondrial functions. Such experiments are in progress.

Supported by grants from the Netherlands Organisation for Pure Scientific Research (ZWO) under auspices of the Netherlands Foundation for Fundamental Medical Research (FUNGO) and the Princess Beatrix Fund (The Hague, The Netherlands). The secreterial help of Truus Klebach and Paula Zwaal is gratefully acknowledged.

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Received August 20, 1986