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Received October 20, 1984 /
Accepted August 10, 1985

Eur J Pediatr (1986) 145:136–138

A prenatal test for the cerebro-hepato-renal (Zellweger) syndrome by demonstration of the absence of catalase-containing particles (peroxisomes) in cultured amniotic fluid cells

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Abstract. In this paper we show that whereas acyl-CoA: dihydroxyacetone phosphate acyltransferase, a membrane-bound peroxisomal enzyme, is deficient in homogenates of cultured amniotic fluid cells of fetuses with Zellweger syndrome, catalase a soluble peroxisomal matrix enzyme is present in normal amounts. Digitonin titration experiments revealed a striking difference in the percentage of particle-bound catalase in control and Zellweger amniocytes: in Zellweger amniocytes all catalase activity was found to be present in the soluble cytoplasm, (<5% particle-bound), whereas in control amniocytes catalase was found to be predominantly particle-bound (62% ± 8%, $n = 5$).

Measurement of the percentage of particle-bound catalase by means of digitonin titrations thus provides a simple prenatal test for Zellweger syndrome via the direct demonstration of the presence or absence of catalase-containing particles (peroxisomes).

Key words: Cerebro-hepato-renal syndrome – Zellweger syndrome – Peroxisomes – Prenatal diagnosis – Inborn error

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Introduction

In 1973 Goldfischer et al. [3] described the absence of morphologically distinct peroxisomes in liver and kidney from patients with the cerebro-hepato-renal (Zellweger) syndrome. Although peroxisomes initially were regarded as having only a modest function in cellular metabolism, recent studies have indicated that peroxisomes are involved in a number of metabolic processes, including ether phospholipid biosynthesis [4, 6], very long chain fatty acid oxidation [10], bile acid synthesis [7] and dicarboxylic acid oxidation [9]. Indeed the absence of peroxisomes in Zellweger syndrome is generally held responsible for the multitude of biochemical abnormalities, such as an accumulation of very long chain fatty acids [1], pipecolic acid [2] and trihydroxycoprostanoic acid [5].

Acyl-CoA: dihydroxyacetonephosphate acyltransferase, a membrane-bound peroxisomal enzyme catalysing the first step in ether phospholipid biosynthesis, is deficient in tissues and

fibroblasts from patients with Zellweger syndrome [11]. Three peroxisomal matrix enzymes (D-amino acid oxidase, L-α-hydroxyacid oxidase and catalase) however were found to be present in near normal amounts in liver homogenates from patients with Zellweger syndrome [13]. Similarly, catalase also was not deficient in cultured skin fibroblasts from the patients. Digitonin titration experiments revealed an aberrant intracellular localization of catalase in fibroblasts from patients with Zellweger syndrome. Whereas catalase was present predominantly in subcellular particles distinct from mitochondria or lysosomes in control fibroblasts, all of the catalase activity in Zellweger fibroblasts was found to be in the soluble cytoplasm.

In this paper we report that catalase also is not deficient in Zellweger amniocytes. As in Zellweger fibroblasts, catalase was found to be fully cytosolic in Zellweger amniocytes, contrary to the situation in control amniocytes in which the bulk of the catalase activity was particle bound. These findings provide a simple prenatal test for Zellweger syndrome using digitonin to demonstrate directly the presence or absence of catalase-containing particles in cultured amniocytes. An advantage of the present method is the requirement of only minimal amounts of amniocytes (100–200 μg protein).

Materials and methods

Enzyme activity measurements. Catalase (H_2O_2 : H_2O_2 oxidoreductase, EC 1.11.1.6) was measured in homogenates of cultured amniotic fluid cells by registering the production of O_2 polarographically at 20°C in a medium containing 50 m*M* potassium phosphate, 10 m*M* sodium perborate and 0.025% (w/v) sodium cholate; the final pH was 7.4.

The activity of acyl-CoA: dihydroxyacetone phosphate acyltransferase (EC 2.3.1.42) was measured exactly as described before [11].

Measurement of subcellular localization of enzymes in cultured amniotic fluid cells. Intact cultured amniotic fluid cells from controls and fetuses with Zellweger syndrome (courtesy Dr. H. W. Moser and Dr. A. E. Moser) were incubated in isotonic sucrose media containing different concentrations of digitonin. The activity of catalase and lactate dehydrogenase was measured exactly as described before [13].

Enzyme sources. Amniotic fluid cells were cultured and harvested according to standard procedures and stored at −80°C or used immediately if intact amniocytes were required for latency measurements. Protein levels were determined according to Lowry et al. [8].

Results

We recently reported that in contrast to acyl-CoA: dihydroxyacetone phosphate acyltransferase, catalase is not deficient in liver and fibroblasts from patients with Zellweger syndrome [13]. The results in Table 1 illustrate the same pattern for cultured amniotic fluid cells: whereas acyl-CoA: dihydroxyacetone phosphate acyltransferase was found to be deficient in homogenates of amniotic fluid cells from two fetuses affected by Zellweger syndrome, as recently reported [12], catalase was found to be present in near normal amounts. It should be stressed that whereas the specific activity of dihydroxyacetone phosphate acyltransferase in cultured fibroblasts or amniocytes from controls was approximately similar, catalase activity in amniocytes was much lower than in fibroblasts (compare [13]).

Table 1. Activities of catalase and acyl-CoA: dihydroxyacetone phosphate acyltransferase in control and Zellweger amniocytes

Amniotic fluid cells	Activity of	
	Catalase (μmol O_2/min per mg)	Acyl-CoA: dihydroxyacetone phosphate acyltransferase (nmol/2 h per mg)
Controls	2.74 ± 0.83 (7)	8.52 ± 2.52 (6)
Zellweger fetus 1	4.2	0.14
Zellweger fetus 2	3.4	0.04

Catalase and acyl-CoA: dihydroxyacetone phosphate acyltransferase were assayed in amniocytes as described in Materials and methods. Results are expressed as mean ± SD with the number of cell lines in parenthesis

The results in Table 1 raise the question of the subcellular localization of catalase in control and Zellweger amniocytes. We recently published a method for investigating the subcellular localization of enzymes within a cell [13]. With this method, which is based upon the use of digitonin selectively to render the different intracellular membranes permeable and the lack of activity of an enzyme due to the presence of an impermeable membrane preventing free accessibility of the substrate to the enzyme, the different intracellular compartments could be visualized. It was found that in control fibroblasts the percentage of particle-bound catalase was 65% ± 8% (mean ± SD; $n = 9$). In fibroblasts from a series of six patients the percentage of particle-bound catalase was in all cases <5%.

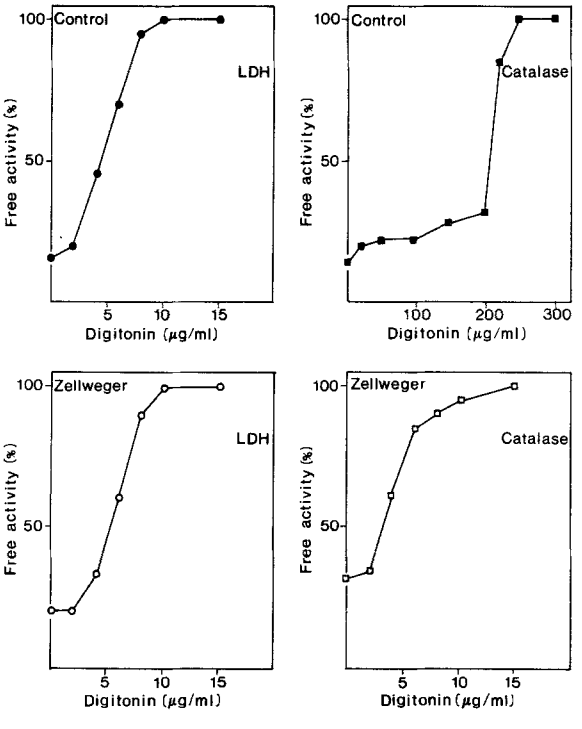

Fig. 1. Measurement of particle-bound catalase in cultured amniotic fluid cells from control and Zellweger fetuses: free activity of lactate dehydrogenase (*LDH*) and catalase as a function of the concentration of digitonin used. Confluent amniocytes were incubated in an isotonic sucrose medium containing the amounts of digitonin indicated and the free activity of lactate dehydrogenase and catalase was measured as described in detail in [13]. Open symbols refer to Zellweger amniocytes, whereas closed symbols refer to control amniocytes

Figure 1 shows the results of a digitonin titration experiment with control and Zellweger amniocytes. The activities of catalase and lactate dehydrogenase were measured as a function of different digitonin concentrations. The amount of digitonin required to release the latency of catalase in control amniocytes was many-fold higher than the amount required to abolish the latency of lactate dehydrogenase, a cytosolic marker enzyme. Thus most of the catalase is particle bound, i.e. present in subcellular organelles (peroxisomes) in control amniocytes. A completely different picture emerged in Zellweger amniocytes (lower part Fig. 1): full activity of catalase was already elicited at very low digitonin concentrations. The results obtained indicate that in contrast to control amniocytes, identical amounts of digitonin were required to abolish the latency of both catalase and lactate dehydrogenase.

Discussion

The results presented in this paper show that catalase is present in near normal amounts in homogenates of cultured Zellweger amniocytes. The data in Fig. 1 indicate that in control amniocytes catalase is present in a subcellular organelle, thus providing evidence for the existence of peroxisomes in amniocytes as supported by the presence of a peroxisomal membrane-bound enzyme, such as acyl-CoA: dihydroxyacetone phosphate acyltransferase. Furthermore, the results in Fig. 1 show that amniocytes from fetuses with Zellweger syndrome lack catalase-containing particles (peroxisomes), since catalase is found to be present in the same compartment as the cytosolic enzyme lactate dehydrogenase. The present results indicate that digitonin titrations immediately reveal the presence or absence of catalase-containing particles, thus providing a simple test for diagnosis of Zellweger syndrome, not only postnatally in cultured fibroblasts but also prenatally in cultured amniocytes. An advantage of the present method is the requirement of low amounts of amniotic fluid cells (100–200 µg protein), thus minimizing the time needed for culture. Use of the digitonin technique described here together with determination of dihydroxyacetone phosphate acyltransferase activity in amniocytes so far has resulted in the diagnosis of four fetuses with Zellweger syndrome. Furthermore, preliminary investigations indicate that the digitonin technique can also be used in chorionic villi fibroblasts, thus allowing prenatal detection of Zellweger syndrome at an early stage.

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Received June 17, 1985 /
Accepted August 23, 1985

Eur J Pediatr (1986) 145:138–141

Effect of splenectomy on destructive bone changes in children with chronic (Type I) Gaucher disease

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Abstract. The incidence and severity of osteolytic bone changes in patients with chronic (Type I) Gaucher disease splenectomized in the first decade of life were compared to those in patients of the same age group and similar degree of severity of the disease in whom the spleen remained intact at least until the second half of the second decade. The size of the spleen, measured by palpation, was used as an index of severity. In the splenectomized group osteolytic changes appeared within a few months following

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Abbreviation: CGD = chronic, non-neuropathic (Type I) Gaucher disease