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Case report

Very large peroxisomes in distinct peroxisomal disorders (rhizomelic chondrodysplasia punctata and acyl-CoA oxidase deficiency): novel data

D. De Craemer[1], M.J. Zweens[2], S. Lyonnet[3], R.J.A. Wanders[4], B.T. Poll-The[5], R.B.H. Schutgens[4], J.J.J. Waelkens[2], J.M. Saudubray[3], and F. Roels[1]

[1] Department of Human Anatomy and Embryology, Vrije Universiteit, Brussels, Belgium
[2] Department of Paediatrics, Catharina Ziekenhuis, Eindhoven, The Netherlands
[3] Clinique du Génétique Médicale, INSERM U-12, Hôpital Necker, Paris, France
[4] Department of Paediatrics, University Hospital, Amsterdam, The Netherlands
[5] Department of Metabolic Diseases, Wilhelmina Kinderziekenhuis, Utrecht, The Netherlands

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Summary. We report very large hepatic peroxisomes (*d*-circle >1 μm) in a patient with rhizomelic chondrodysplasia punctata and a patient with acyl-CoA oxidase deficiency. The effects of peroxisomal enlargement on the enzymatic activity are discussed. As increase in peroxisomal size is also reported in at least 12 other patients with peroxisomal disorders, we propose a relationship between the enlargement of the organelles and their functional deficiency.

Key words: Morphometry – Cytochemistry – Catalase – Thiolase – Neonatal adrenoleukodystrophy

Introduction

Peroxisomal disorders reveal a heterogeneity of clinical symptoms and peroxisomal morphology. Enlarged hepatic peroxisomes have been reported in some 12 patients with peroxisomal acyl-CoA oxidase deficiency (AOX deficiency), rhizomelic chondrodysplasia punctata (rCPD), NALD-like syndromes, and peroxisomal thiolase deficiency (pseudo-Zellweger) (Heymans et al. 1986; Naidu et al. 1988; Poll-The et al. 1988; Roels et al. 1988; Hughes et al. 1990; Kyllerman et al. 1990; Espeel et al. 1991). This suggests a causal relationship between enlargement of the organelles and enzymatic deficiency.

We report on the size of hepatocellular peroxisomes in two novel cases.

Offprint requests to: D. De Craemer, Menselijk Anatomie en Embryologie, Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels, Belgium

Materials and methods

Patient 1 is an 8-month-old boy with rCDP. The diagnosis was based on skeletal radiography and the near complete absence of plasmalogens in erythrocytes. Patient 2 was an 18–19 weeks fetus (post-menstrual age). The mother had earlier given birth to two children with AOX deficiency (Poll-The et al. 1988). Western blots revealed the absence of all three components of the acyl-CoA oxidase protein in amniocytes. C26:0 levels and the C26/C22 ratio were elevated in amniotic fluid. After termination of pregnancy, the absence of acyl-CoA oxidase antigen was confirmed in liver.

Liver specimens of both patients and of controls were stained for catalase activity with diaminobenzidine (Roels et al. 1987). The morphometry of the peroxisomes was examined on random, calibrated electron micrographs using a semi-automated system as described elsewhere (De Craemer et al. 1991). The size of the organelles is expressed by the parameter *d*-circle (i.e. the diameter of the circle with the same area as the measured profile).

Results

Light microscopic evaluation of the liver of the AOX-deficient patient was difficult because of numerous lipofuscin granules and weak peroxisomal staining. In the rCDP specimen, very large peroxisomes were observed. In sections of adjacent parenchymal cells, however, peroxisomes were few or absent. Electron microscopy revealed the presence of enlarged peroxisomes in both patients (Fig. 1): some organelles had a *d*-circle higher than 1.5 μm (Table 1).

In the rCDP specimen, a lower concentration of catalase reaction product was found in the larger peroxisomes. The number (N_V) and surface density (S_V) of the peroxisomes were decreased. However, volume density (V_v) was normal because of the larger peroxisomal diameter.

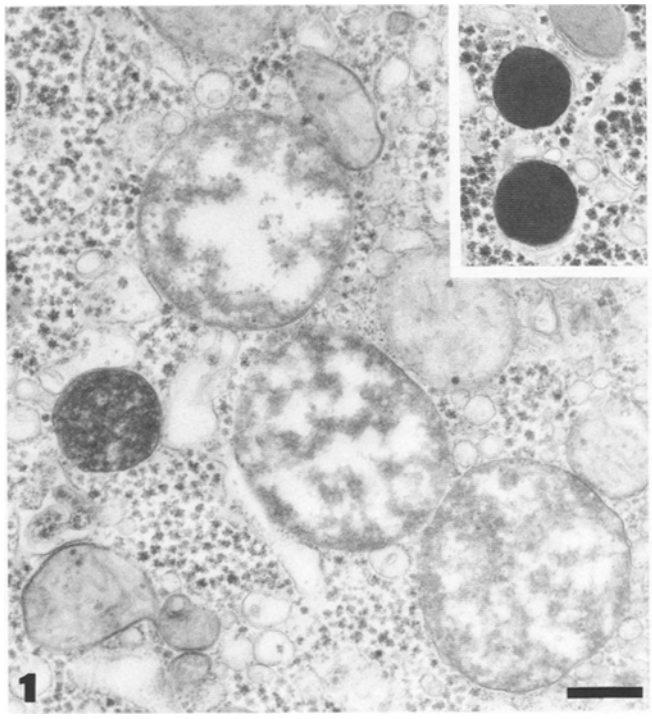

Fig. 1. Liver of patient 1 (rhizomelic chondrodysplasia punctata): three enlarged peroxisomes reveal a diameter of ±1.4 μm and contain little reaction product. Catalase stain, ×20000. *Bar*: 0.5 μm. *Inset*: 4-month-old control. Peroxisomes reveal a homogeneous electron density. ×20000

The AOX-deficient specimen and one control fetal liver of 16 weeks showed post-mortem alterations and membrane discontinuities. Peroxisomal matrix material was clumped and mainly in contact with the membrane, and the mean peroxisomal diameter was increased when compared to normal fetal peroxisomes. However, in the AOX-deficient liver a relative stronger increase in peroxisomal diameter was found when compared to the control fetus of 16 weeks (Table 1). This reflects the in vivo alteration. Three percent of the organelles in the AOX-deficient case revealed on axial ratio smaller than the lowest control value, that is to say, they were more elongated (not shown). V_v and S_v were increased and N_v was normal (Table 1).

Discussion

Heymans et al. (1986) reported large, irregularly shaped peroxisomes in one patient with rCDP. This was not found in liver specimens of two other patients (Poulos et al. 1988). However, no morphometry was performed in either of the studies. Enlarged peroxisomes were found earlier in AOX deficiency in two children born to the same family as our case (Poll-The et al. 1988; Roels et al. 1988). Elongation (minimal axial ratio: 0.241) was observed in one case (Poll-The et al. 1988).

In addition to these patients, at least nine other peroxisomal disorders with enlarged hepatic peroxisomes have been documented (Naidu et al. 1988; Roels et al. 1988; Hughes et al. 1990; Kyllerman et al. 1990; Espeel et al. 1991 – reviewed in Roels et al. 1991). Peroxisomal metabolism in vivo is determined by the amount of enzymes present in the organelles, but will also be related to the import of substrates and the export of reaction products. Even competition by other substrates may decrease the activity in the living cell (Wanders et al. 1987; Boles et al. 1991). In addition, during the process of turnover of the peroxisomal constituents, the newly synthesized as well as the obsolete molecules must pass across the membrane. Thus, changes in membrane area will influence the import, activity and turnover of the peroxisomal enzymes (Roels 1991). Roels and Cornelis (1989) showed that the concentration of catalase activity in individual organelles was inversely related to their radius.

Table 1. Morphometric results

Patients	Diameter (*d*-circle) (μm)			V_v (%)	N_v (μm^{-3})	S_v (μm^{-1})	*n*
	Mean ± SEM	corr[a]	max				
Adults							
6 controls	0.529 ± 0.020	0.648	0.898	1.06	0.096	0.108	872
Infants							
control 6 weeks	0.445 ± 0.013	0.555	0.848	0.70	0.128	0.085	117
control 4 months	0.529 ± 0.010	0.650	1.027	1.18	0.110	0.131	141
patient 1 (rCDP)[b]	0.719 ± 0.028	0.918	1.862	1.04	0.043	0.077	97
Fetuses							
3 controls 16 weeks	0.388 ± 0.018	0.476	0.679				417
1 control 16 weeks	0.481 ± 0.015 (+24%)	0.572 (+20%)	0.888				102
2 controls 17–20 weeks	0.518 ± 0.006	0.629	0.806				203
patient 2[c] (AOX deficiency)[d]	0.701 ± 0.027 (+35%)	0.905 (+44%)	1.608	1.76	0.092	0.162	109

[a] Corrected for sectioning effect
[b] Rhizomelic chondrodysplasia punctata
[c] With post-mortem alterations
[d] Acyl-CoA oxidase deficiency

When peroxisomes are compared to spheres, the volume of one single organelle is linearly related to the radius cubed while its surface area is linearly related to the radius squared. This implies that larger organelles have a smaller surface area to volume ratio. This is unfavourable for the exchange of metabolites over the peroxisomal membrane. Although larger organelles possess more enzymatic capacity, the latter are enclosed by relatively less membrane when compared to normal-sized or smaller peroxisomes. Peroxisomal enzymes may not fully use their capacity in enlarged organelles when the exchange over the peroxisomal membrane becomes a limiting factor.

An augmentation of the metabolic efficiency is achieved by an increase in surface area to volume ratio. This is accomplished by an increased number of smaller organelles. If we consider peroxisomes as spheres, the total membrane area of two equally sized daughter organelles is the cube root of two times larger (+26%) than the membrane area of the mother organelle with the same volume. In many conditions, an induction of peroxisomal β-oxidation is accompanied by proliferation of the peroxisomes and a simultaneous decrease in size (Christiansen et al. 1981; Fringes and Reith 1982; Sherratt et al. 1982; Kerckaert et al. 1989; Veitch et al. 1989). These changes also implicate an increased S_v.

In patient 2, S_v was increased although a peroxisomal enzyme was deficient. This suggests a defect of the import machinery in addition to the compartmentation factor which prevents enzymatic functioning at a maximal rate.

Enlargement might also be a phenomenon secondary to the enzyme deficiency and subsequent enhanced metabolic load, such as increased serum very long chain fatty acids.

Our novel data strongly suggest that the concurrence of functional deficiency and peroxisomal enlargement may not be fortuitous, but causally related, and diagnostically significant.

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