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Is the cytosolic catalase induced by peroxisome proliferators in mouse liver on its way to the peroxisomes?

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Dietary treatment of male C57B1/6 mice with clofibrate, nafenopin or WY-14.643 resulted in a modest (at most 2-fold) increase in the total catalase activity in the whole homogenate and mitochondrial fraction prepared from the livers of these animals. On the other hand, the catalase activity recovered in the cytosolic fraction was increased 12- to 18-fold, i.e. 30–35% of the total catalase activity in the hepatic homogenate was present in the high-speed supernatant fraction after treatment with these peroxisome proliferators. A study of the time course of the changes in peroxisomal and cytosolic catalase activities demonstrated that the peroxisomal activity both increased upon initiation of exposure and decreased after termination of treatment several days after the increase and decrease, respectively, in the corresponding cytosolic activity. This finding suggests that the cytosolic catalase may be on its way to incorporation into peroxisomes.

Catalase; Peroxisome proliferation; Clofibrate; Nafenopin; WY-14.643; Liver; Mouse

1. INTRODUCTION

Despite many years of study, there is still a great deal of controversy concerning the subcellular localization of catalase (which plays an important role in the detoxication of H_2O_2 by transforming this reactive oxygen species to water) in mammalian cells (e.g. [1,2]). Catalase is thought to be primarily localized in peroxisomes, but there are also numerous indications that a small portion of the total activity may also be present in the cytoplasm under normal physiological conditions (e.g. [1,3–6]).

After treatment of rodents with certain peroxisome proliferators, the catalase activity in the high-speed supernatant (cytosolic) fraction from the liver has been found to be increased (e.g. [1,7–9]).

It has been argued that this increase reflects leakage from peroxisomes during the subfractionation procedure [10] and an immunocytochemical analysis concluded that catalase is entirely localized in the peroxisomes of mouse hepatocytes, both without and after treatment with clofibrate [11]. The criticism of the latter study is that the total volume of the peroxisomes is much smaller than that of the cytoplasm and that there might therefore be a much higher concentration of catalase in the peroxisomes, which would thus appear to contain all of this enzyme. In addition, when we permeabilized the plasma membrane of isolated mouse hepatocytes with digitonin under conditions which left intracellular organelles intact, 5–10% of the total catalase in control cells and 30–40% of this activity in hepatocytes from clofibrate-treated animals was released to the surrounding medium [9].

Therefore, it is our working hypothesis that peroxisome proliferators selectively increase the cytosolic content of catalase in mouse hepatocytes. The object of the present study was to characterize this increase by clofibrate, nafenopin and WY-14.643 in more detail. In addition, the time-course of changes in catalase upon treatment with clofibrate and subsequent termination of this exposure were investigated in order to determine whether there was a temporal 'precursor–product' relationship between cytosolic and peroxisomal catalase in this system.

2. EXPERIMENTAL

2.1. *Chemicals*

Clofibrate (ethyl *p*-chlorophenoxyisobutyrate) (ICI, Macclesfield, Cheshire, England) and hydrogen peroxide (30%) (E. Merck, Darmstadt, Germany) were purchased from the sources indicated. Nafenopin (2-methyl-2(*p*(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy)proprionic acid) (Dr. Charles A. Brownley, CIBA Pharmaceutical Do., Summit, NJ, USA) and WY-14.643 (4-chloro-6-(2,3- xylidino)2-pyrimidinyl-thio)acetic acid) (Ms. Kay Marley, Wyeth Laboratories Inc., Philadelphia, PA, USA) were kind gifts from the individuals and organizations indicated. All other chemicals were of analytical grade and obtained from common commercial sources.

2.2. *Animals and treatment*

Male C57B1/6 mice (ALAB, Sollentuna, Sweden) weighing approximately 20 g at the commencement of the experiment were employed

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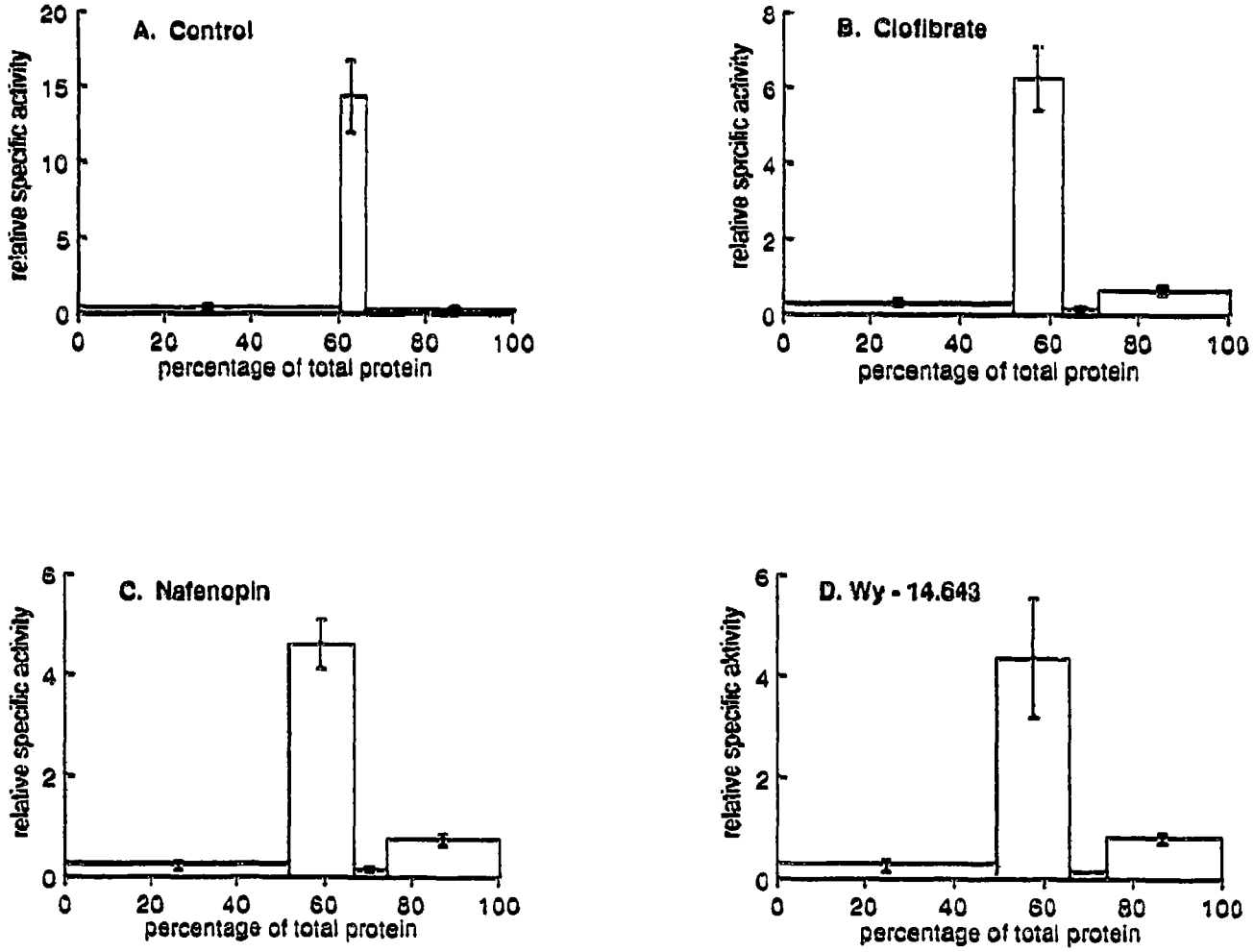

Fig. 1. deDuve plots of the distribution of catalase in the livers of control mice and of mice exposed to clofibrate, nafenopin or WY-14.643. After treatment of animals, subcellular fractionation and assay of catalase as described in section 2. deDuve plots were constructed in the usual fashion, with the fractions being, from left to right, the nuclear, mitochondrial, microsomal and cytosolic fractions. The values are means for 3 different animals and the bars represent S.D. The specific activities in the whole homogenate were 25, 41, 41 and 36 μmol hydrogen peroxide consumed per min per mg protein in untreated, clofibrate-, nafenopin and WY-14.643-treated mice, respectively.

Table I

Subcellular distribution of catalase activity in mouse liver after peroxisome proliferation with clofibrate, nafenopin or WY-14.643

Peroxisome proliferator	Catalase activity (% of control)[a]			
	Whole homogenate	Peroxisomes[b]	Cytosol	$\frac{\text{Cytosol}}{\text{Homogenate}} \times 100$
None (control)	100	100	100	5.6
Clofibrate	173 ± 24**	203 ± 37**	1320 ± 210***	31.0
Nafenopin	209 ± 35**	198 ± 12**	1820 ± 360***	35.0
WY-14.643	199 ± 11**	197 ± 21**	1170 ± 160***	33.0

The values represent the means ± standard deviations for 3 different animals.
**$P<0.01$; ***$P<0.001$ compared to the control group, according to Student's t-test.
[a]Activities expressed as mmol hydrogen peroxide consumed per g liver were compared. Corrections were not made for incomplete recovery in the subfractions.
[b]Measured in the mitochondrial fraction, which contains 50% of the total peroxisomes, both in control and treated animals [12,13].

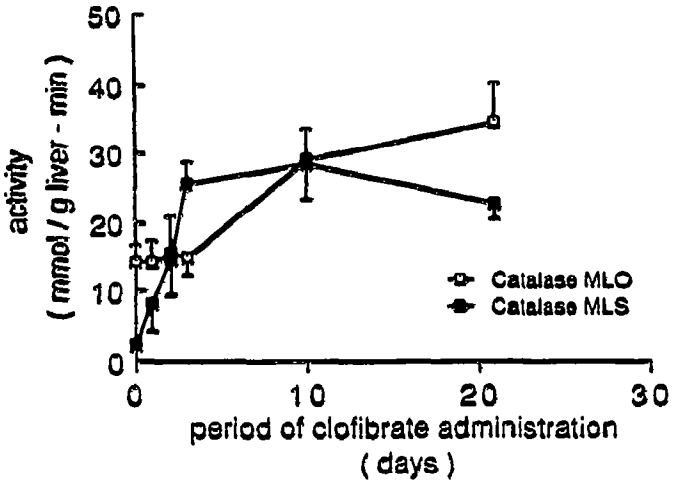

Fig. 2. Time-courses of the increases in peroxisomal (MLO) and cytosolic (MLS) catalase activities in the livers of mice exposed to clofibrate in their diet. After treatment of the animals for different periods of time up to 20 days, subcellular fractionation was performed and catalase activities in the mitochondrial (MLO) and high-speed supernatant (MLS) fractions assayed as described in section 2. The values are means for 3 different animals and the bars represent standard deviations.

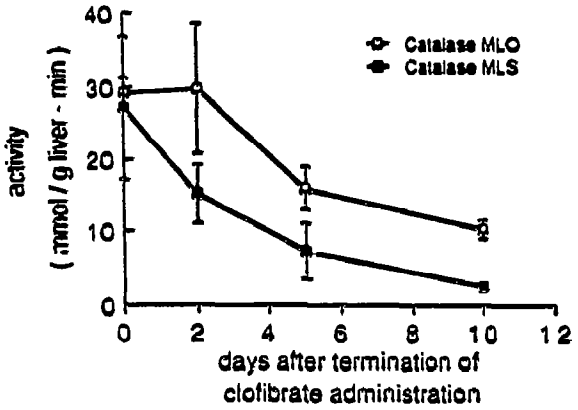

Fig. 3. Time-courses of the return of peroxisomal (MLO) and cytosolic (MLS) catalase activities in the livers of mice to control levels after termination of exposure to clofibrate. After dietary exposure of the mice to clofibrate for 20 days and subsequent recovery for various periods of time, subcellular fractionation was performed and catalase activities in the mitochondrial (MLO) and high-speed supernatant (MLS) fractions assayed as described in section 2. The values are means for 3 different animals and the bars represent S.D.

in this study. The treated animals received 0.5% (w/w) clofibrate, 0.125% (w/w) nafenopin or 0.125% (w/w) WY-14.643 mixed with powdered standard laboratory chow (Ewos, Södertälje, Sweden) for 4 days before sacrifice by cervical dislocation. In one experiment the animals were treated for 20 days with the same dose of clofibrate and thereafter allowed to recover for 10 days.

2.3. *Subcellular fractionation*

After sacrifice, the liver was removed and freed from the gallbladder. This tissue was homogenized in 2 vols. ice-cold 0.25 M sucrose using 4 up-and-down strokes of a Potter-Elvehjem homogenizer at 400 rpm. After dilution to 1 g liver/5 ml with sucrose, differential centrifugation was employed to subfractionate this homogenate into nuclear (600 × g_{av} pellet), mitochondrial (10,000 × g_{av} pellet), microsomal (105,000 × g_{av} pellet) and cytosolic (105,000 × g_{av} supernate) fractions, as described and characterized previously [12].

2.4. *Peroxisomal catalase*

Peroxisomal catalase activity was assayed in the mitochondrial fraction (which contains 50% of the total hepatic peroxisomes, both in control and in treated animals [12,13]) on the basis of spectrophotometric monitoring of the disappearance of H_2O_2 [14].

2.5. *Protein*

Protein was determined by the procedure of Lowry and coworkers [15], using bovine serum albumin as the standard.

2.6. *deDuve plots*

deDuve plots were constructed in the usual fashion [16].

3. RESULTS AND DISCUSSION

Table I documents the changes observed in hepatic catalase activity in mouse treated with clofibrate, nafenopin or WY-14.643. All of these peroxisome proliferators caused an approximately 2-fold increase in the total homogenate and peroxisomal catalase activities, but a much more dramatic increase in the corresponding activity recovered in the high-speed supernatant fraction. This much more pronounced effect on the cytosolic catalase results in the recovery of 30–35% of the total catalase in the high-speed supernatant after treatment with the peroxisome proliferators (Table I). This redistribution of activity is also apparent from the deDuve plot presented in Fig. 1, where the relative specific activity of catalase in the mitochondrial fraction (containing 50% of the peroxisomes) is dramatically decreased and the area under the bar representing the cytosolic fraction dramatically increased after exposure.

Fig. 2 illustrates the time-course for the increase in peroxisomal and cytosolic catalase activities during treatment of mice with clofibrate for 20 days. It can be seen that the maximal increase in the cytosolic activity is achieved in about 3 days, whereas the increase in peroxisomal catalase does not even begin until 3 days after commencement of treatment and requires about 10 days to reach the maximal value.

Fig. 3 shows the time-course for the return of peroxisomal and cytosolic catalase activities to control levels after termination of exposure to clofibrate for 20 days. Here, it is apparent that the cytosolic activity is reduced by about 50% within 2 days of recovery and returns to control levels within 10 days. The peroxisomal activity, on the other hand, does not begin to decrease until at least 2 days after cessation of treatment, but thereafter returns to the control level relatively rapidly.

Thus, upon exposure of mice to clofibrate the hepatic cytosolic catalase activity increases prior to the increase in peroxisomal activity and upon termination of this exposure, the cytosolic activity also decreases first. This

may indicate that the cytosolic activity is translocated to peroxisomes, a suggestion which is particularly interesting in the light of a recent report that the peroxisomes initially formed in response to treatment of mice with clofibrate lack catalase [17]. Also of relevance in this respect is the report that during peroxisomal biogenesis in regenerating liver, the membrane is synthesized first and the matrix proteins synthesized and incorporated thereafter [18]. In vitro studies on the import of cytosolic catalase from the livers of clofibrate-treated mice into peroxisomes are now in progress.

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