The organelle pathology and demonstration of mitochondrial superoxide dismutase deficiency in two patients with Dubin–Johnson–Sprinz syndrome

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Summary

1. Liver biopsies from two patients with the Dubin–Johnson–Sprinz syndrome were subjected to analytical subcellular fractionation by sucrose-density-gradient centrifugation and enzymic microassays.

2. A selective deficiency of mitochondrial superoxide dismutase has been demonstrated in these patients.

3. The significance of this enzyme deficiency is discussed in relation to the organelle pathology of the syndrome.

Key words: Dubin–Johnson Syndrome, free radicals, hyperbilirubinaemia, liver disease, lysosomes, melanin, mitochondria, superoxide dismutase.

Introduction

The Dubin–Johnson–Sprinz syndrome is an inherited form of conjugated hyperbilirubinaemia in which there is a progressive accumulation of both lipofuscin and melanin in the hepatocytes. Distinct enzyme defects are likely to underlie this syndrome as it is familial, probably with autosomal recessive inheritance (Shani, Seligsohn & Adam, 1973; Edwards, 1975). We have used analytical subcellular fractionation procedures in combination with marker enzyme microassays to study the hepatic organelle pathology in two patients with this syndrome, and have found a deficiency of mitochondrial superoxide dismutase.

Methods and patients

Detailed histories of the two patients investigated (D.C. and B.A.) are given by Seymour, Neale & Peters (1977). Analytical subcellular fractionation, and enzymic and protein assays were performed as described before (Seymour, Neale & Peters, 1974; Peters, 1976; Seymour & Peters, 1977). Superoxide dismutase was estimated as described by Beauchamp & Fridovich (1971) with Ontosein (Diagnostic Data Inc., Mountain View, Calif., U.S.A.) as a source of standard enzyme. Serum manganese was measured by atomic absorption spectrophotometry (Grafflage, Buttgereit, Kubler & Mertens, 1974). The studies were approved by the local Ethical Committee.

Results

Table 1 shows the activities of some marker enzymes for the various organelles of the liver tissue in biopsies from the two patients and from seven control subjects. Apart from the threefold increase in the activity of the lysosomal enzyme N-acetyl-β-glucosaminidase and a small increase in the activities of the three plasma membrane marker enzymes, alkaline phosphatase, 5'-nucleotidase and leucyl-2-naphthylamidase, there is no significant difference between the patient and control groups. In particular, the activities of total superoxide...
TABLE 1. Enzyme activities and protein content of liver biopsy specimens from patients with Dubin–Johnson–Sprinz syndrome and from control subjects

Control activities are expressed as mean value ± SE with the number of specimens assayed given in parentheses. Enzyme activities are expressed as munits/mg of protein (superoxide dismutase is expressed as ng of enzyme standard/mg of protein). Protein is expressed as total (mg) of biopsy studied. Control data are from Seymour & Peters (1977) and from C. A. Seymour & T. J. Peters (unpublished results).

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Control activities</th>
<th>Patient D.C.</th>
<th>Patient B.A.</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>EC no. 1.15.1.1</td>
<td>2.88</td>
<td>3.19</td>
<td>1.92 ± 0.01 (7)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>1.1.1.37</td>
<td>2900</td>
<td>2720</td>
<td>3000 ± 310 (4)</td>
</tr>
<tr>
<td>Catalase</td>
<td>1.1.1.6</td>
<td>270</td>
<td>280</td>
<td>248 ± 27 (5)</td>
</tr>
<tr>
<td>N-Acetyl β-glucosaminidase</td>
<td>3.2.1.30</td>
<td>7.55</td>
<td>6.64</td>
<td>2.03 ± 0.28 (36)</td>
</tr>
<tr>
<td>Neutral α-glucosidase</td>
<td>3.2.1.20</td>
<td>0.694</td>
<td>1.021</td>
<td>0.60 ± 0.06 (36)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>3.1.3.1</td>
<td>2.55</td>
<td>2.89</td>
<td>1.35 ± 0.17 (35)</td>
</tr>
<tr>
<td>5'-Nucleotidase</td>
<td>3.1.3.5</td>
<td>18.3</td>
<td>20.9</td>
<td>13.7 ± 2.4 (37)</td>
</tr>
<tr>
<td>Leucyl-2-naphthylamidase</td>
<td>3.4.11.1</td>
<td>3.98</td>
<td>3.69</td>
<td>1.28 ± 0.17 (37)</td>
</tr>
<tr>
<td>γ-Glutamyl transpeptidase</td>
<td>2.3.2.2</td>
<td>7.18</td>
<td>5.12</td>
<td>5.08 ± 0.81 (35)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>1.1.1.27</td>
<td>500</td>
<td>301</td>
<td>345 ± 31 (5)</td>
</tr>
<tr>
<td>Protein (mg)</td>
<td>—</td>
<td>1.20</td>
<td>1.01</td>
<td>1.2 ± 0.3 (5)</td>
</tr>
</tbody>
</table>

dismutase in the two patients are within the control range.

Fig. 1 compares the distribution of the various organelle marker enzymes in the sucrose density gradients when subjected to analytical subcellular fractionation. Acid phosphatase and particularly N-acetyl-β-glucosaminidase show differences, with a significant decrease in lysosomal median density.

There is no significant difference in the distribution of relative amounts of soluble and particulate malate dehydrogenase between the patients and control subjects. The equilibrium density of the mitochondria, 1.19, is identical in the two groups. However, superoxide dismutase does show differences. In the control specimens most of the activity is due to soluble enzyme remaining in the starting layer but there is a distinct peak (5–10% of total activity) corresponding to the position of particulate malate dehydrogenase. In both patients this latter component is absent whereas there was a distinct mitochondrial peak, in all five control studies.

The plasma membrane enzymes, 5'-nucleotide, γ-glutamyl transpeptidase and alkaline phosphatase (not shown) show little difference between the two groups but there is a significant decrease in the median density of the endoplasmic reticulum marker, neutral α-glucosidase. Catalase, a marker for the peroxisomes, shows a greater proportion of soluble to particulate enzyme for the patients compared with the control subjects, but these differences are probably not significant. The serum manganese was 0.098 in patient D.C. and 0.051 μmol/l in patient B.A. (normal range 0.0091–0.109 μmol/l).

Discussion

Our results demonstrate a deficiency of the mitochondrial component of superoxide dismutase in liver biopsy tissue from two patients with the Dubin–Johnson–Sprinz syndrome. Superoxide dismutase has a dual intracellular localization, with a major cytosol and a minor mitochondrial component (Peeters–Joris, Vandevoorde & Baudhuin, 1975; Tyler, 1975). Approximately 10–15% of activity is mitochondrial, with distinct forms of the enzyme located in the matrix and the intermembranous space of the mitochondria (Weisiger & Fridovich, 1973; Panchenko, Brusov, Gerasimov & Loktaeva, 1975). The major mitochondrial dismutase is a Mn²⁺-dependent enzyme (Fridovich, 1975) but the normal serum manganese concentrations, though not necessarily reflecting tissue values in our patients, would indicate that deficiency of manganese is not responsible for this syndrome.

We have discussed the significance of the lysosomal change previously (Seymour et al., 1977), but it was not then clear why pigments accumulate in excessive amounts in this syndrome. The selective deficiency of mitochondrial superoxide dismutase can now explain many of the features of this syndrome. Free radicals, particularly $\text{O}_2^-$, are intermediates and byproducts of many intracellular reactions (Bors, Saran, Lengfelder, Spöttl...
Organelle pathology in Dubin–Johnson syndrome

FIG. 1. Subcellular fractionation by isopycnic centrifugation of postnuclear supernatants from control subjects (-----) and two patients with Dubin–Johnson–Sprinz syndrome (---). Patient D.C. was studied on two occasions. Graphs show frequency-density distributions for the various enzymes. Frequency (+ SD) is defined as the fraction of total recovered activity present in the individual fraction divided by the density span covered. The activity over the density 1-05-1·10 represents the enzyme remaining in the starting layer. The percentage recovered activity (mean ± SD) for the patients are: acid phosphatase, 81 ± 2; N-acetyl-β-glucosaminidase, 71 ± 3; malate dehydrogenase, 73 ± 3; superoxide dismutase, 100 ± 10; 5′-nucleotidase, 70 ± 2; γ-glutamyl transpeptidase, 75 ± 6; α-glucosidase, 105 ± 2; catalase, 82 ± 4. The control data are the mean distribution from five to eight experiments with recovered enzyme activities of 83–97%.

& Michel, 1974; Fridovitch, 1974) including some of those occurring within mitochondria (Forman & Kennedy, 1974; Dionisi, Galeotti, Terranova & Azzi, 1975). The formation of lipid peroxides and thus of lipofuscin pigments is one product of the interaction of free radicals with the fatty acid components of membrane lipids (Dillard & Tappel, 1971; Fong, McCay, Poyer, Keele & Misra, 1973; Högberg, Orrenius & O’Brien, 1975; Kellogg & Fridovitch, 1975). Superoxide dismutase will inhibit the formation of these lipid peroxides and thus a deficiency of the enzyme would be expected to lead to the accumulation of excessive lipofuscin in mitochondria. Morphological studies have also suggested a role for the mitochondria in the formation of these pigments (Woods, DuBuy, Burk & Hesselbach, 1949; Colocolough, Hack, Helmy, Vaughn & Veith, 1972).

These organelles have a relatively short life span with a half-life in rat liver of approximately 10 days (Fletcher & Sanadi, 1961; Beattie, Bashford & Koritz, 1967; Ashwell & Work, 1970). It is highly likely that the degradation of effete mitochondria is largely due to lysosomal autophagy and digestion (Swift & Hruban, 1964; de Duve & Wattiaux, 1966; Glaumann, Berezovsky, Ericsson & Trump, 1975). Lipids and particularly lipofuscin are relatively undegradable and this latter pigment would therefore accumulate in excessive amounts in this organelle, and it is known that this accumulation of pigment increases as the patients get older (Barone, Inferrera & Carrozza, 1969), or in mutant Corriedale sheep (Cornelius, Arias & Osburn, 1965) and howler monkeys (Katz, Gilardoni, Genovese, Wilkininski, Cornelius & Malinow, 1968) with this syndrome.

The source of the melanin is not clear. The biosynthesis of pigment is complex and ill-understood (Swan, 1974), and involves autoxidation steps which may be catalysed by O2 radicals (Kumar, Ravindranath, Vaidyanathan & Rao, 1972; Miller & Rapp, 1973). Isolated melanin has been shown to contain a high amount of free radicals (Longuet-Higgins, 1960; Mason, Ingram & Allen, 1960; Lukiewicz, 1972) and it is possible that the melanin is synthesized as an alternative free radical scavenging agent in the absence of the mitochondrial superoxide dismutase.

It is not clear how the enzyme deficiency is related to the impairment of conjugated bilirubin excretion. It is possible that free radial-mediated damage affects other intracellular organelles, although the activity of cytosol superoxide dismutase is normal. Ashworth & Sanders (1960) have suggested that lysosomes play a role in the biliary excretion of bilirubin conjugates and it would be expected that this function would be impaired if the lysosomes were distended with pigments.

The demonstration (Ben Ezzar, Blonder, Shani, Seligsohn, Post, Adam & Szeinberg, 1973) that there is an abnormal urinary excretion pattern of
porphyrins in Dubin–Johnson syndrome has led Arias (1973) to postulate a defect in an intracellular haem protein responsible for bilirubin glucuronide excretion. However, the hepatic activity of the enzyme involved in the synthesis of these porphyrins has recently been shown to be normal (Shimizu, Kondo, Kuchiba & Urate, 1977). It is of interest, however, that certain steps in porphyrin biosynthesis occur within the mitochondrion. Finally, it is noteworthy that free radicals have been implicated in DNA cleavage (Harman, 1962; van Hemmer & Mealing, 1975; Morgan, Cone & Elgert, 1976) and Edwards (1975) has noted an unusually high incidence of malignancy in the Dubin–Johnson syndrome.

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References


