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Short Communication

Smith–Lemli–Opitz syndrome: Deficient Δ^7 -reductase activity in cultured skin fibroblasts and chorionic villus fibroblasts and its application to pre- and postnatal detection

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The Smith–Lemli–Opitz syndrome (SLO) is a recently identified disorder of cholesterol biosynthesis, clinically characterized by typical facial dysmorphism including microcephaly, ptosis, cataracts, anteverted nares, low-set posteriorly rotated ears and micrognathia, multiple congenital limb and organ anomalies and severe mental retardation with incomplete myelination of the brain and peripheral nerves (Smith et al 1964). In 1993, Irons and coworkers (1993) reported the marked elevation of 7-dehydrocholesterol in patients with SLO, suggesting a defect in the penultimate step of cholesterol biosynthesis (Tint et al 1994). This was established experimentally by the same group of investigators, who reported deficient 7-dehydrocholesterol Δ^7 -reductase activity in microsomes prepared from liver specimens obtained from four SLO homozygotes (Shafer et al 1995). These experiments were followed by studies in cultured skin fibroblasts using radiolabelled lathosterol as a more stable precursor (Honda et al 1995). Similar experiments were performed by Lund and coworkers (1996) also using [³H]lathosterol.

In this paper we describe a simple method allowing direct measurement of 7-dehydrocholesterol Δ^7 -reductase activity in cultured skin fibroblasts and chorionic villus fibroblasts using radiolabelled 7-dehydrocholesterol which is now commercially available.

MATERIALS AND METHODS

Synthesis of 7-dehydrocholesterol: [4-¹⁴C]-7-Dehydrocholesterol was prepared from [4-¹⁴C]cholesterol as follows: a mixture of [4-¹⁴C]cholesterol (Amersham, UK) and unlabelled cholesterol was first converted into the corresponding cholesterol acetate using

acetyl chloride and triethylamine in dry dichloromethane. The [4- 14 C]cholesterol acetate was then reacted with 1,3-dibromo-5,5-dimethylhydantoin in hexane by irradiation (500 W photolamp) for 1h to form the 7-bromo derivative. Subsequently the 7-bromo derivative was converted into [4- 14 C]dehydrocholesterol acetate with quinaldine in xylene. After purification by preparative TLC, the acetate was finally converted into [4- 14 C]-7-dehydrocholesterol by saponification. Specific activity was 20mCi/mmol.

Culture materials: Dulbecco's modified Eagle's medium (DMEM) was obtained from BioWhittaker, Verviers, Belgium; fetal calf serum (FCS) from Bodinco, Alkmaar, The Netherlands; delipidated fetal calf serum (DFCS) and Hepes from Boehringer Mannheim, Germany; Ham's F10 medium and trypsin-EDTA from Gibco BRL, Paisley, UK; penicillin and streptomycin from Imperial Laboratories, Andover, UK; culture flasks and 6-well cluster plates from Costar, Zwanenburg, The Netherlands; cholesterol and 7-dehydrocholesterol from Sigma, Zwijndrecht, The Netherlands; argentated TLC plates from Alltech, Breda, The Netherlands. All other chemicals were purchased from Merck (Darmstadt, Germany) as analytical grade reagents.

BM 15.766 was a kind gift from Dr Joh. Pill (Boehringer Mannheim, Germany). A phosphoimager was used to visualize the radioactive spots on the TLC plates (type 400B, Molecular Dynamics, Sunnyvale, CA, USA).

Cell lines and cell culture: Skin fibroblasts were obtained from patients with unrelated metabolic disorders and from a patient suffering from SLO. Chorionic villus fibroblasts were obtained from pregnancies at risk of an unrelated metabolic disorder.

Skin fibroblasts were cultured (150cm² culture flasks) in DMEM containing 10% (v/v) FCS, penicillin (100IU/ml) and streptomycin (100µg/ml) at 37°C in a humidified CO₂ atmosphere (5%).

To measure the conversion of 7-dehydrocholesterol to cholesterol in monolayers of fibroblasts, cells were trypsinized and transferred to 6-well cluster plates and cultured in DMEM as described previously (Malle et al 1995). Prior to the experiments, preconfluent cells were washed with phosphate-buffered saline (PBS, pH 7.4) and further incubated for 24h in DMEM containing 10% (v/v) DFCS. Subsequently the cells were incubated for 4h in a regular incubator in Ham's F10 fortified with Hepes (pH 7.4) to maintain the pH. BM 15.766 (10µmol/L) was added to two wells to mimic a Δ^7 -reductase deficiency. Next the substrate, [14 C]-7-dehydrocholesterol (50nmol; 3.7kBq), was added to each well and the cells were incubated for 48h in a regular incubator.

After incubation the cells were washed twice with PBS and subsequently treated as described by Malle et al (1995). After extraction with chloroform-2-propanol (2:1, v/v) lipids were dried under N₂ and dissolved in 100µl chloroform and applied to the argentated TLC plates. The plates were developed twice in n-heptane-ethyl acetate (2:1, v/v). The plates were allowed to dry in between. The radioactive spots were visualized using a phosphor screen and scanned on a phosphoimager. The images obtained were quantified with the ImageQuant computer program (version 3.3).

Case history: The patient, a boy was born as the fourth child of healthy, nonconsanguineous parents. The first three children are healthy. The pregnancy was complicated by

unexplained growth retardation. At birth he was microcephalic and had a high nasal bridge, a long philtrum, broad dental ridges, schisis of the soft palate, retrognathia, a small penis with chorda and bilateral syndactyly of the second and the third toes. On the basis of the dysmorphic features the presence of the Smith–Lemli–Opitz syndrome was suspected, which was confirmed by gas chromatography of plasma, demonstrating a decreased cholesterol level and an increased 7-dehydrocholesterol level.

RESULTS

When cultured skin fibroblasts were incubated in a medium containing radiolabelled 7-dehydrocholesterol, virtually all of the compound was converted into a compound with elution characteristics identical to a cholesterol standard run in parallel, as shown in Figure 1A. To discover whether conversion of 7-dehydrocholesterol to cholesterol was, indeed, brought about by 7-dehydrocholesterol Δ^7 -reductase, the same experiment was repeated but in the presence of 10 $\mu\text{mol/L}$ BM 15.766, a known inhibitor of the microsomal Δ^7 -reductase, at least in rats (Pill et al 1985; Aufenanger et al 1986). Figure 1B shows that inclusion of BM 15.766 in the incubation medium completely blocks formation of the product, thus suggesting the direct involvement of Δ^7 -reductase.

With fibroblasts of an SLO-patient, showing all the clinical and biochemical hallmarks reported for SLO (elevated 7-dehydrocholesterol, low cholesterol in plasma), there was virtually no conversion of 7-dehydrocholesterol to cholesterol (Figure 1C).

Figure 1D shows efficient formation of cholesterol from 7-dehydrocholesterol in chorionic villus fibroblasts. In these cells also, BM 15.766 blocks formation of cholesterol almost completely (Figure 1E).

DISCUSSION

We describe a relatively simple method allowing direct measurement of 7-dehydrocholesterol Δ^7 -reductase in cultured skin fibroblasts and chorionic villus fibroblasts. The availability of such a method is especially important since radiolabelled 7-dehydrocholesterol is now commercially available.

We always perform experiments in the presence and absence of BM 15.766, a known inhibitor of Δ^7 -reductase, in order to mimic a deficiency of the Δ^7 -reductase in each patient's cell line under study. The finding that chorionic villus fibroblasts also metabolize 7-dehydrocholesterol efficiently in a BM 15.766-inhibitable fashion suggests that the present method, as well as chromatographic analysis (Mills et al 1996) can be used for prenatal diagnosis. We have so far applied the method to two at-risk pregnancies, which are still in progress.

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Figure 1 Results of 7-dehydrocholesterol Δ^7 -reductase measurements in control fibroblasts (A,B), fibroblasts from a SLO-patient (C), and chorionic villus fibroblasts (D,E). In panels (B) and (E) results are shown of incubations in the presence of 10 $\mu\text{mol/L}$ BM 15.766. Vertical axis: intensity of the radioactive spot. Horizontal axis: relative mobility on TLC plate

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