INCREASE IN STEROL SYNTHESIS AND DECREASE IN CHOLESTEROL EFFLUX IN NIEMANN-PICK DISEASE TYPE C FIBROBLASTS

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INTRODUCTION

Niemann-Pick disease is an inborn error of metabolism characterized by a deficiency in sphingomyelinase activity and sphingomyelin storage in the A and B types. In the C type (NPC), cholesterol is often accumulated to a greater extent than sphingomyelin, and the primary defect is, at the present time, unknown. In previous studies, we pointed out close relationships between sphingomyelin and cholesterol metabolisms in human fibroblasts¹,², and we demonstrated that NPC fibroblasts are able to degrade exogenous sphingomyelin³. We also suggested that in NPC disease, the primary defect could concern cholesterol rather than sphingomyelin metabolism². Recently, a deficiency in cholesterol esterification has been described in NPC cells by Pentchev et al.⁴,⁵. In the present study, we investigated different aspects of cholesterol metabolism in 3 NPC patients fibroblasts: sterol synthesis, esterification and efflux.

MATERIALS AND METHODS

Cell culture

Control fibroblasts were obtained by skin biopsy performed in the laboratory on healthy adult subjects. Fibroblasts from NPC patients were obtained by skin biopsy performed in the laboratory for SB and Z lines, or purchased from the Human Genetic Mutant Cell Repository (Camden, USA) for the GM 110 cell line. The three cell lines exhibited partial deficiency in sphingomyelinase activity (about 25-30%, 30-35% and 50% of controls for SB, GM 110 and Z cells, respectively). Cells were maintained in Ham F10 medium (Flow) supplemented with 10% foetal calf serum (Gibco) in 25 cm² Falcon flasks, at 37°C, in a 5% CO₂ humidified atmosphere.
Lipid synthesis from sodium acetate

Cells were preincubated 24h in medium supplemented either with 10% foetal calf serum (low rate of sterol synthesis) or 10% lipoprotein-deficient human serum (high rate of sterol synthesis). [1-14C]-sodium acetate (Commissariat à l'Energie Atomique, Saclay, France, 48 mCi/mmol, 25µCi/ml) was introduced and incubation performed 24h. Cells were washed 4 times with a phosphate-buffered solution pH 7.4, then harvested with rubber policemen, and lipid analysis was performed by thin layer chromatography after application of an aliquot of the cell suspension on silica-gel plate, according to Mazière et al. After autoradiography, the radioactivity of sterols was measured by liquid scintillation in an Intertechnique instrument. Results are expressed in pmol of precursor incorporated per mg of cell protein.

Oleic acid incorporation

[1-14C]-oleic acid (Amersham, 52 mCi/m mole) was evaporated to dryness under nitrogen, then resuspended in 0.2mg/ml fatty acid-free serumalbumin. The final concentration was 1 µCi/ml of Ham F10 medium supplemented with 10% foetal calf serum, and incubation with the cells was performed during 4h at 37°C. After extensive washing, cells were harvested with rubber policemen and lipid analysis performed by thin layer chromatography as above. Results are expressed in pmol of 14C-oleic acid incorporated per mg of cell protein.

Cholesterol esterification and cholesterol efflux

[1-14C]-cholesterol (Amersham, 55 mCi/m mole, 2.5µCi/ml) was introduced in the culture medium in ethanolic solution. The final concentration of ethanol in the culture medium was 0.5%. For the study of cholesterol esterification, cells were incubated 24h with 14C-cholesterol, then harvested and the radioactivity of cholesterol and cholesteryl esters determined after thin layer chromatography. Results are expressed in pmol of cholesterol incorporated per mg of cell protein. For the study of cholesterol efflux, cells were preincubated 24h with [1-14C]-cholesterol 2.5µCi/ml. After washing 4 times with a phosphate-buffered solution pH 7.4 supplemented with 0.2% bovine serum albumin, cells were incubated with non-labeled medium (chase). After harvesting, the radioactivity of cholesterol was determined after separation by thin layer chromatography as described above. Results, calculated in pmol of cholesterol per mg cell protein, are expressed as percentages of the value found at the beginning of the chase period.

RESULTS

As it has been demonstrated by Brown et al. 7 that exogenous cholesterol supply by the low-density-lipoprotein pathway results in repression of the key enzyme Hydroxy-Methylglutaryl Coenzyme A reductase, sterol synthesis from 14C-sodium acetate was comparatively studied in the presence of LDL (10% foetal calf serum) or in their absence (10% lipoprotein-deficient serum). As shown in Table 1, in complete medium, sterol synthesis was markedly increased in NPC cells as compared to control (x 3 for GM110 and x 4.5 for S.B.). When cells were preincubated in a medium devoid of lipoproteins, sterol synthesis was greatly enhanced, as expected, but this phenomenon is less marked in NPC (x 3) than in control cells (x 5-8).