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Changes in cholesterol metabolism in cultured fibroblasts from patients with Niemann-Pick disease

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SUMMARY

Cultured skin fibroblasts from 6 patients with Niemann-Pick disease type A were investigated for cholesterol metabolism. An increase in cholesterol synthesis from ^{14}C-sodium acetate was observed in all cases. A decrease in ^{14}C-oleic acid incorporation into cholesteryl esters was found in 5 of 6 cases. ^{125}I-low density lipoprotein binding was significantly reduced in 3 of 4 investigated cases.

INTRODUCTION

Niemann-Pick disease (N.P. disease) is an inborn error of metabolism characterized in type A and B by a severe dificiency in sphingomyelinase activity (1). The correlated storage of sphingomyelin observed in cells from reticuloendothelial system is generally accompanied by an increase in free cholesterol content (1). Several observations suggested that it exists a close relationship between the metabolism of sphingomyelin and cholesterol. In the lens cortex membrane, with aging (2), or in atherosclerotic coronary arteries (3), an increase in both sphingomyelin and free cholesterol was observed. Demel & col. (4) by differential scanning calorimetry showed a preferential affinity of cholesterol for sphingomyelin. We recently described (5) an *in vitro* inhibition of sphingomyelinase by free cholesterol, probably related to a change in the physical state of the substrate . At last, Gatt & col. (6) pointed out a decrease in ^{125}I-low density lipoprotein binding and an increase

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in cholesterol biosynthesis in cultured human skin fibroblasts feeded with positively charged liposomes containing sphingomyelin.

In this paper we present the results of our observations concerning the metabolism of cholesterol (biosynthesis from ^{14}C-sodium acetate, esterification by ^{14}C-oleic acid, and binding of ^{125}I-LDL) in cultured fibroblasts from 6 patients with N.P. disease type A. Cultured fibroblasts from controls and patients with other metabolic diseases (Fabry's disease, fucosidosis and cystinosis) were comparatively studied.

MATERIALS AND METHODS

Cell culture : N.P. fibroblasts were obtained by skin biopsy from 3 patients identified (clinical feature and sphingomyelinase activity) as N.P. disease type A (B., Kh., P.), or from the Human Genetic Mutant Cell Repository, Camden, N.J., USA (GM_{112}, $GM_{370}$and GM_{559}). Control fibroblasts and fibroblasts from patients with Fabry's disease and familial hypercholesterolemia were obtained by skin biopsy. Fibroblasts from patients with fucosidosis and cystinosis were graciously furnished by Dr. Hösli (Pasteur Institute, Paris). Cells were cultured in 25 cm^2 Corning flasks containing 5 ml of Ham F_{10} medium (Flow) supplemented with 15 % foetal calf serum (Gibco). All experiments were carried out on confluent cells.

Synthesis of cholesterol from [2-^{14}C] sodium acetate : [2-^{14}C] sodium acetate 48 mCi/mmole, CEA, France, was introduced in the culture medium at the concentration of 10 µCi/ml. Incubation was performed during 4 h at 37°C. Cells were then washed 4 times with 5 ml of a phosphate buffered solution, harvested with a rubberpoliceman, centrifuged and resuspended in NaCl 9 °/₀₀. Protein determination was done by the method of Lowry. Lipid analysis was performed as previously described (7). Results are expressed in cpm x 10^{-3}/mg of cell protein.

Incorporation of [1-^{14}C] oleic acid into cholesteryl esters : after evaporation do dryness under N_2, [1-^{14}C] oleic acid 52 mCi/mmole (CEA, France), was resuspended in a fatty acid-free human serumalbumin solution 0.2 mg/ml. The final concentration was 0.5 µCi/ml of medium, and the incubation performed for 4 h at 37°C. Cells were then washed 4 times with a phosphate buffered solution, and harvested with a rubberpoliceman. Protein determination was done by the method of Lowry , and lipid analysis performed as previously described (7). Results are expressed in cpm x 10^{-3}/mg of cell protein.

^{125}I-LDL binding : LDL were prepared as previously described (8), and labelled with ^{125}I following Mac Farlane (9). ^{125}I-LDL binding to cultured fibroblasts was studied by the method of Goldstein and Brown (10). Specific binding is expressed in cpm x 10^{-3}/mg of cell protein.

Table I : Incorporation of [2-^{14}C] sodium acetate into cholesterol. [2-^{14}C] sodium acetate 48 mCi/mmole was introduced in the culture medium at the concentration of 10 µCi/ml. Cells were confluent. Incubation was performed 4 h at 37°C. Lipids were analysed by thin layer chromatography as previously described (see Materials and methods). Results are expressed in cpm x10^{-3}/mg of cell protein. Range in brackets.

Fibroblast strain		Number of experiments	Incorporation of [2-^{14}C] sodium acetate into cholesterol (cpmx10^{-3}/mg protein)
Controls	T_1	5	3.8 (2.6-5.1)
	T_2	4	4.9 (3.6-6.4)
Niemann-Pick disease	B.	6	13.2 (10.7-14.5)
	Kh.	5	11.2 (8.5-14.6)
	P.	3	12.3 (10.4-14.3)
	GM_{112}	2	8.0 (6.1-9.9)
	GM_{370}	3	9.1 (7.6-10.2)
	GM_{559}	2	7.8 (5.5-10.1)
Fabry's disease	La	3	5.0 (3.9-6.0)
Fucosidosis	GM_{802}	2	3.7 (3.0-4.4)
Cystinosis	Ben	3	4.3 (3.1-5.2)

RESULTS AND DISCUSSION

In Table I it can be seen that the biosynthesis of cholesterol from ^{14}C-sodium acetate was 1.8 to 3 fold increased in fibroblasts from patients with N.P. disease, and not significantly modified in other mutants. Table II shows that the incorporation of ^{14}C-oleic acid into cholesteryl esters was markedly decreased in 5 cases of N.P. disease (B.,Kh.,P., GM_{112} and GM_{370}) and only slightly decreased in GM_{559}. No significant difference was found between controls and other studied mutants. Table III presents the results of ^{125}I-LDL binding by fibroblasts from 4 patients with N.P. disease, controls, and one case of familial hypercholesterolemia (homozygote) which was comparatively studied for testing the method of ^{125}I-LDL binding. ^{125}I-LDL binding was notably lowered in 2 cases of N.P. disease (B. and Kh., respectively 48 % and 60 % of controls), slightly decreased in one case (GM_{112}, about 70 % of controls), and not significantly

Table II : Incorporation of [1-^{14}C] oleic acid into cholesteryl esters. [1-^{14}C] oleic acid 52 mCi/mmole was resuspended in a fatty acid-free human serumalbumin solution 0.2 mg/ml. The final concentrations was 0.5 µCi/ml of medium. Confluent cells were incubated for 4 h at 37°C, and lipid analysis performed as previously described (see Materials and methods). Results are expressed in cpm x 10^{-3}/mg of cell protein. Range in brackets.

Fibroblast strain		Number of experiments	Incorporation of [1-^{14}C] oleic acid into cholesteryl esters (cpmx10^{-3}/mg protein)
Controls	T_1	4	34.2 (25.6-42.9)
	T_2	3	28.5 (20.9-35.3)
Niemann-Pick disease	B.	4	13.3 (9.5-17.2)
	Kh.	4	17.6 (13.6-19.9)
	P.	3	16.8 (13.9-20.8)
	GM_{112}	2	19.2 (17.8-20.6)
	GM_{370}	2	21.0 (16.6-25.4)
	GM_{559}	2	27.8 (23.2-32.4)
Fabry's disease	La	3	38.0 (32.8-41.7)
Fucosidosis	GM_{802}	2	27.4 (23.4-31.4)
Cystinosis	Ben.	2	29.1 (22.7-35.5)

modified in the last case studied (GM_{559}). In contrast, as it could be expected, ^{125}I-LDL binding was dramatically reduced in cells from familial hypercholesterolemia.

These results are consistent with the high incidence of the free cholesterol accumulation observed in N.P. disease. Several mechanisms could be involved in this phenomenon. The data of Gatt & col. (6) demonstrated that an intracellular accumulation of sphingomyelin artificially induced by feeding normal human fibroblasts with positively charged liposomes containing sphingomyelin produced a decrease in LDL binding and an increase in cholesterol biosynthesis. These authors suggested that the binding of sphingomyelin to intracellular cholesterol could result in a loss of the regulatory effect of cholesterol on its own metabolism. Such a phenomenon induced by sphingomyelin accumulated in cultured N.P. fibroblasts could explain our observations,

Table III : ^{125}I-LDL binding by cultured fibroblasts from controls, Niemann-Pick disease and familial hypercholesterolemia. Cells were confluent. ^{125}I-LDL (about 6.10^8 cpm/mg protein) were prepared following Mac Farlane, and the binding carried out as described by Goldstein and Brown (see Materials and methods). Specific binding is expressed in cpm x 10^{-3}/mg of cell protein. Range in brackets.

Fibroblast strain		Number of experiments	^{125}I-LDL binding (cpmx10^{-3}/ mg protein)
Controls	T_1	3	46.7 (38.3-52.6)
	T_2	3	38.6 (34.2-45.1)
Niemann-Pick disease	B.	3	20.5 (16.9-24.2)
	Kh.	3	25.6 (19.7-29.2)
	GM_{112}	2	29.8 (27.3-32.3)
	GM_{559}	2	36.0 (31.6-40.4)
Familial hypercholesterolemia	Li.	3	2.2 (1.8-2.6)

as it has been demonstrated that it exists a considerable increase in sphingomyelin content in cultured cells from patients with N.P. disease (11, 12). At last, the decrease of ^{14}C-oleic acid incorporation into cholesteryl esters by N.P. fibroblasts could be related to the decrease of LDL binding by these cells, as the Acyl CoA : cholesterol Acyl transferase is known to be induced by LDL (13).

However, the present results have to be confirmed by studies on a larger scale, as it must be considered that the metabolism (and especially cholesterol metabolism)of cultured fibroblasts is highly dependent on culture conditions.

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