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STIMULATION OF SPHINGOMYELIN HYDROLYSIS BY CANNABIDIOL IN FIBROBLASTS FROM A NIEMANN-PICK PATIENT

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The hydrolysis of sphingomyelin in cells derived from a Niemann-Pick patient was studied using both a labelled precursor and measurement of endogenous levels. In vitro exposure of the cells to cannabidiol resulted in a large decrease in both the relative and absolute amounts of this lipid; the drug had a smaller effect on normal fibroblasts. Cannabidiol has been tested in the clinic as an antiepileptic agent with some success; our findings suggest that it may also be useful in relieving the symptoms associated with Niemann-Pick disease.

The effects of Niemann-Pick disease are generally thought to be due to decreased sphingomyelinase activity in the affected individual which stem from a specific genetic defect (1). Research progress in this area has centered around better defining this biochemical lesion and developing reliable early diagnostic tests which can be utilized for genetic counselling purposes (2). Therapeutic approaches involving enzyme replacement have been attempted (2) however, none of these procedures has been successfully applied in the clinic as yet.

The approach described in this report utilizes observations from our laboratory on the lipid mobilizing properties of the cannabinoids (3,4,5). The most well known member of this group of substances is Δ^1-tetrahydrocannabinol, however, several of the non-psychoactive cannabinoids also show potent effects on lipid hydrolysis (3). We hypothesized that cells with higher than normal lipid content would be particularly sensitive to the cannabinoids and that this might form the basis for a chemotherapeutic approach to Niemann-Pick disease.

A convenient model used by others (6-12) in this area for various types of studies involves the culturing of fibroblasts obtained from Niemann-Pick

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patients. These cells exhibit the increased levels of sphingomyelin and cholesterol which are typical of tissues derived from such patients and show low hydrolytic activity toward exogenous substrate as well (8-11). We report here our results on the effects of cannabinoids on phospholipid levels in a model of this type.

METHODS

The Niemann-Pick cells were obtained from the Institute for Medical Research, Camden, N.J. The cells were characterized as fibroblasts and were designated as repository number GM 0370. The cells were grown to near confluency as monolayers in "miniwells" using Eagle's minimum essential medium supplemented with 15% fetal calf serum. The normal cells were WI-38 human lung fibroblasts grown under the same conditions as the GM 0370 cells (see also refs. 3,4). The cannabinoids were supplied by the National Institute on Drug Abuse, Rockville, MD; the chlorpromazine was purchased from Sigma and the alfaxalone was from Steraloids, Inc. Wilton, N.H. (N-Methyl-^{14}C) sphingomyelin was purchased from Amersham and had a specific activity of 60 Ci/mol.

Cells were extracted with 95% ethanol (2 x 1 ml. per monolayer) and thin layer chromatographic analysis was performed on silica gel G plates as described by us previously (4). The hplc was performed on a Waters instrument equipped with a Kratos detector and a Rainin Microsorb Si 5μ column (250x4.6 mm). The eluent was a mixture of acetonitrile:methanol:0.001 N H_3PO_4 (130:5:1.5) and the flow rate was 1.5 ml/min. Under these conditions standards of lecithin and sphingomyelin showed elution times of 18 and 36 minutes respectively.

RESULTS

The uptake of (N-methyl-^{14}C) sphingomyelin by the Niemann-Pick fibroblasts ranged from 50-75% of the added radioactivity. This is somewhat higher than that reported for a similar procedure (8), however, those authors had attempted to introduce larger amounts of labelled lipid. When the labelled cells were given a 24 hr. exposure to the various agents listed in Table I, a dose and structure dependent shift of the label from sphingomyelin to lecithin was observed. Cannabidiol was the most effective substance tested while THC and chlorpromazine showed smaller effects. The steroid anaesthetic, alphaxalone, was essentially inactive.

Endogenous levels of lipids from normal human fibroblasts were measured by hplc analysis (Table II) and compared with the lipid levels in Niemann-Pick cells measured under identical conditions (Table III). In the normal cells, cannabidiol did not produce a significant decrease in sphingomyelin, however, a highly significant 77% reduction was found in the Niemann-Pick cells. The

TABLE I

STIMULATION OF SPHINGOMYELINASE ACTIVITY IN NIEMANN-PICK CELLS*

Conditions	Ratio of Lecithin/Sphingomyelin†
Control‡	4.2
Cannabidiol (10 μg/ml)	14.2
Cannabidiol (20 μg/ml)	47.1
Δ^1-Tetrahydrocannabinol (20 μg/ml)	6.8
Chlorpromazine (100 μg/ml)	7.9
Alphaxalone (100 μg/ml)	4.9

*The cells were grown as described in METHODS and labelled by exposure to 28,000 dpm (N-methyl-^{14}C) sphingomyelin in ethanol for 24 hrs. The lecithin and sphingomyelin contents were then analyzed by tlc (see METHODS).

†Ratio of dpms in each tlc zone x 10^{-3}.

‡Vehicle (10 λ ethanol). All drugs were added in 10λ ethanol.

cannabidiol-induced decrease in lecithin was identical in both cell types indicating that phospholipase A_2 activity is normal in the Niemann-Pick cells. Cannabidiol was about 3.5 times more effective in lowering sphingomyelin than in lowering lecithin in the affected cells suggesting some degree of selectivity under these conditions.

TABLE II

EFFECT OF CANNABIDIOL ON THE LIPID CONTENT OF NORMAL FIBROBLASTS*

	Control†	Cannabidiol†	Change(%)	p‡
Lecithin	1.80 ± 0.26	1.42 ± 0.07	-21	<0.005
Sphingomyelin	0.40 ± 0.5	0.33 ± 0.06	-17	>0.05
Ratio	4.50	4.33		

*WI-38 human lung fibroblasts grown as described in METHODS. Cells were exposed to the drug or vehicle for 30 mins and analyzed by hplc (see METHODS). Values are areas under the peaks in arbitrary units obtained by measuring light absorption at 203 nm.

†The control consisted of a 30 min. exposure to vehicle (10 λ ethanol). Cannabidiol was added in 10 ethanol to give a final concentration of 5 μg/ml (16 μM).

‡Data is given as the mean of three measurements ± S.D. p was determined by the Student's t test.

TABLE III

EFFECT OF CANNABIDIOL ON THE LIPID CONTENT OF NIEMANN-PICK FIBROBLASTS*

	Control†	Cannabidiol†	Change(%)	p‡
Lecithin	2.64 ± 0.70	2.09 ± 0.10	-21	<0.005
Sphingomyelin	3.01 ± 1.25	0.70 ± 0.14	-77	<0.0005
Ratio	0.88	2.99		

*As in Table I.

†As in Table I.

‡As in Table I.

DISCUSSION

Our data strongly suggest that the excess lipids stored in Niemann-Pick cells can be mobilized by cannabinoids. The evidence presented here was derived by two independent procedures thereby increasing the reliability of our conclusion. The data in Table I were obtained by labelling the cells with (N-methyl-^{14}C) sphingomyelin and measuring the transfer of the choline moiety to lecithin presumably by the "salvage pathway". Tables II and III were obtained by measuring total lipid content of the cells utilizing hplc separation and light absorption at 203 nm for detection.

A limited structure-activity study is also contained in the data in Table I. All of the substances tested are believed to interact with cellular lipids (13-15), however, cannabidiol seems to show a greater potency in these cells and, possibly, greater specificity for sphingomyelin. The data from this very brief study do not allow any mechanistic conclusions to be made, however, it seems likely that cannabidiol may act by altering the physical state of the sphingomyelin making it more sensitive to hydrolysis by the lower-than-normal sphingomyelinase activity present. On the other hand, at this point we can not entirely rule out a direct effect on the enzyme.

An obvious question is whether cannabidiol would show a similar effect when tested in vivo. There are reports in the literature which would justify

in vivo studies in both animals and patients. For example, cannabidiol has been tested in the clinic and shown to be an effective antiepileptic agent (16). There are also data on experimental animal models related to epilepsy which support the human studies (17,18). An attractive property of cannabidiol is its low toxicity and lack of psychoactivity even in orally administered doses up to 600 mg/day (19).

The particular cell line utilized in this report was derived from a Type A Niemann-Pick patient (20) representing the most extreme form of the disease (1). It has been reported that these cells show less than 3% of the normal sphingomyelinase activity (9) making this model a particularly challenging test for any lipid mobilizing agent. It would seem that cannabidiol, or a similar substance, offers a new approach towards the treatment of Niemann-Pick patients particularly in view of the lack of alternatives.

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