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Increased Dolichol Content in Glucocorticoid-Sensitive Human T-Cell Leukemia Line Grown in the Presence of Dexamethasone

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Two cell lines, derived from human T-cell acute leukemia, one glucocorticoid sensitive (CEM-C7) and the other glucocorticoid resistant (CEM-C1) were grown in the presence of 1 x 10^{-7} M dexamethasone and were analyzed for their dolichol content. After 24 hrs of incubation, dolichols became significantly elevated in the sensitive but not in the resistant line. Lovastatin, the specific inhibitor of cholesterol synthesis did not affect dolichol levels in either of the two cell lines. The results raise the possibility that dolichol accumulation might be involved in the early stages of the glucocorticoid-induced apoptosis (directed cell killing).

The inhibition of growth and the destruction of lymphoid cells by glucocorticoids is a receptor-dependent process which consists of a series of biochemical and morphological events, collectively known as apoptosis. In its early stages, apoptosis is characterized by the condensation of nuclear chromatin followed by fragmentation of nuclear DNA at internucleosomal linkers and terminating, eventually, in cell death (1).

We have shown previously that another early event which takes place in cultured lymphoid cells of neoplastic origin, treated with glucocorticoids, is the inhibition of acetate incorporation into the non-saponifiable lipid fraction, consisting mainly of cholesterol (2). Significantly, the cell line derived from human acute T-cell leukemia (CEM-C7) used in these studies was shown to depend on de novo synthesis of cholesterol even in the presence

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Abbreviations: Dex, dexamethasone, 9α fluoro-11β, 17α, 21 trihydroxy-16α-methyl-1-4 pregnadiene-3,20-dione; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A.

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of the serum supplemented media (2). Exogenous cholesterol esters present in low density lipoprotein when added to the cultures of these cells, were stored in the cholesterol ester compartment and could not be used as a source of free cholesterol, possibly because of low cholesterol ester hydrolase activity (3).

The present study was undertaken in order to examine the cellular content of dolichols, another mevalonate product which is derived by most tissues from de novo synthesis (4). We have shown previously in HeLa cells that synthesis of dolichols becomes elevated in response to glucocorticoid treatment during growth (5). The demonstration of increased dolichol content in highly sensitive lymphoid cells derived from acute human leukemia and the absence of such dolichol increase in a line which is glucocorticoid resistant could provide information about the role of this group of isoprenoid compounds in the functional integrity of various cell membrane components and their participation in the apoptotic cascade, a regulated cell killing which has been already demonstrated in thymocytes (6,7) and in mouse (8) and human (9) cell lines of lymphoid derivation.

MATERIALS AND METHODS

Cells: The cultures of CEM-C1 and the CEM-C7 cells were given to us by Dr. E.B. Thompson of the University of Texas Medical Branch at Galveston. The detailed procedures for the cultivation of these cells were described in an earlier paper (3). For the experiments, both the Dex-resistant CEM-C1 cells and the Dex-sensitive CEM-C7 cells were grown in RPMI40 medium supplemented with 10% fetal bovine serum inactivated by heating for 30 min at 56°C. The logarithmically growing cells were then changed to serum free Iscove's Medium and incubated in the presence of 10^{-7} M Dex or the equivalent volume of ethanol. The initial cell density varied between experiments from 1 x 10^5 cells/ml to 1 x 10^6 cells/ml. After 24 or 48 hrs of incubation the cells were harvested by centrifugation, washed with phosphate-buffered 0.85% NaCl and the cell pellets were frozen at -10°C until ready for analysis.

Chemicals: All routine chemicals were purchased from Sigma Chemical Co., St. Louis, MO. The media were obtained from Gibco, Grand Island, NY and the fetal bovine serum from Hyclone, Logan, UT. Lovastatin (mevinolin) was donated by Mr. Alfred A. Alberts from the Merck Institute for Therapeutic Research, Rahway, NJ.

Analytical Procedure: Dolichols containing 17-22 isoprene units were isolated from cultures of CEM-1 and CEM-7 cells and quantitated by HPLC. The analytical procedure was a modification of the method reported by Freeman et al. (10). Briefly, the instrumentation consisted of a Beckman (Model 110A) single piston pump which delivered the mobile phase consisting of a mixture of isopropanol and methanol (80:20, v:v) at a rate of 1.0 ml/min. All solvents used were of HPLC grade (J.T. Baker, Phillipsburg, NJ). A Model 7125 Rheodyne injector (Cotati, CA) with a 200 μl loop and a Rheodyne column inlet filter with 0.5 μ pore size were used to introduce prepared samples. The analytical column was 100 x 4.6 mm packed with 3 μm C_{18} particles (Rainin Co., Woburn, MA). A Spectroflow 773 absorbance detector (Kratos, Ramsey, NJ) operated at 210 nm wavelength and Hewlett Packard, Model 3380A integrator were used to obtain chromatograms at a chart speed of 0.5 cm/min and attenuations of 8 and 16.

A stock standard solution was prepared by dissolving 10 mg of porcine liver dolichols (Sigma Chemical Co., St. Louis, MO) in hexane to give a concentration of 100 μg/ml. Analytical standards were prepared in hexane to give standards of 50, 75, 100, 150, 200, 500 and 800 ng/ml. The internal standard solution was prepared by dissolving 2.0 mg of dolichol C_{125} (obtained from Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland) in hexane to give a concentration of 2 μg/ml. All standards were stored below 0°C. The analytical standards and the cell pellets were saponified for one hour in 15% KOH in ethanol. Ten (10.0) ml of petroleum ether (E.M. Science, Cherry Hill, NJ) was dispensed into each tube. Each tube was vortexed for 1.0 min followed by centrifugation. The organic phase (top layer) was then separated using pasteur pipets and passed through previously activated Spice silica gel cartridges (Analtech, Newark, DE). The collected organic phase in culture glass tubes was then evaporated under nitrogen and the sides of the tubes wetted with 250 μl of mobile phase to redissolve the dried residue. After centrifugation, 100-200 μl of this was chromatographed. Dolichol levels were calculated by using the following equation:

$$\text{Areas of } (C_{90} + C_{95} + C_{100} + C_{105})/(C_{125})$$

RESULTS

Two chromatograms showing the distribution of dolichols in extracts from CEM-C7 cells grown without and with Dex are represented in Fig. 1A and 1B respectively. The most common isoprenologues were identified by their retention times as compared to the standards of the known chain length. The first major peak, the C-90 dolichol, could not be completely separated from the large solvent front. Nevertheless, all the peaks were sharp enough to permit quantitation by the method of peak/area ratio. The calibration curve for the dolichols in the range of 50-200 ng was plotted by linear regression which showed a correlation coefficient of 0.996 with good linearity over the entire range. The mean recovery was 89.4% with the standard deviation of

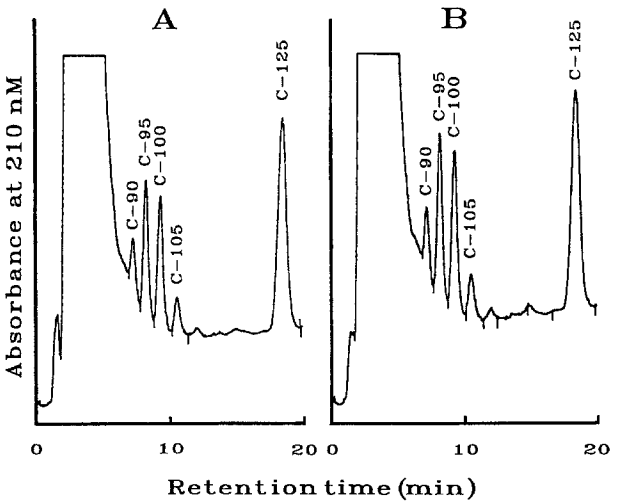

Figure 1. Chromatograms of dolichols extracted from CEM-C7 cells grown for 24 hrs in the absence (A) and in the presence of 10^{-7} M dexamethasone (B). Numbers above peaks indicate numbers of carbon atoms for each isoprenlogue.

8.02%. The amounts of total dolichols were corrected for total protein in the pellet prior to extraction. Nevertheless, variations were obtained between the basal levels of dolichols in individual experiments which could not be traced to any differences in the procedure or in protein content of the cultures. To assess the significance of the tendency of dolichols to increase after treatment with Dex, a series of six consecutive experiments was carried out in which CEM-C1 and CEM-C7 cells were incubated in the presence of 1 x 10^{-7} M Dex and their dolichol contents were compared after 24 and 48 hrs of incubation. The results were analyzed by the Student's t-test and are summarized in Table I. It is evident that there was a statistically significant increase in dolichol content in CEM-C7 cells but not in the Dex-resistant CEM-C1 cells. Although the increase in dolichols after 48 hrs of incubation could have been magnified by a corresponding decrease in protein which becomes significant after 48 hrs of growth in Dex, such decreases were not evident after 24 hrs. Also, the increase in dolichols was, on the average, greater than the decrease in protein.

TABLE 1

Effects of dexamethasone on dolichol levels in human T-cell derived acute leukemia lines CEM-C7 and CEM-C1

Cell Line	Exper.#	24 hr incubation			48 hr incubation		
		Dolichols (μg/mg protein)		Statistics	Dolichols (μg/mg protein)		Statistics
		Control	Dex ($1x10^{-7}$ M)		Control	Dex ($1x10^{-7}$ M)	
CEM-C7	1	2.8	6.7	t = 2.375	9.9	96.9	t = 4.724
	2	4.5	8.6		2.9	13.7	
	3	9.1	14.2	P = 0.063	6.9	43.1	P = 0.006
	4	6.0	8.3		5.6	56.8	
	5	7.4	8.3		10.0	61.4	
	6	24.4	22.7		6.6	56.7	
CEM-C1	1	5.7	8.9	t = 1.221	4.9	7.7	t = 2.201
	2	18.5	19.7		3.4	2.9	
	3	3.6	4.3	p = 0.277	6.3	13.6	P = 0.078
	4	7.4	8.3		9.9	12.4	
	5	6.4	6.8		5.2	12.4	
	6	18.8	17.1		3.4	2.9	

Cell growth and dolichol analysis described in Materials and Methods. All experiments were done on different days. The results were analyzed by the Student's t-test for paired samples.

In addition to the already mentioned decrease in cholesterol synthesis, the Dex-sensitive cells usually show a decrease in the activity in HMG CoA synthase but the main rate limiting enzyme in the mevalonate pathway viz. HMG CoA reductase remains unaffected or even becomes elevated (2,11,12). In order to evaluate these effects in terms of their relationship to the dolichol content of both cell lines, an experiment was done in which, in addition to Dex, we included lovastatin (mevinolin) a specific inhibitor of HMG CoA reductase. The results (Fig. 2) show that lovastatin, in contrast to Dex, did not have any significant effect on dolichol levels in either CEM-C7 or CEM-C1 cells. This observation indicates that Dex might influence dolichol synthesis at a post-mevalonate step possibly involving isopentenyl pyrophosphate (IPP) and/or farnesyl pyrophosphate (FPP) (13).

At least two mechanisms may be proposed for the involvement of Dex in the apoptotic process as it relates to metabolic products of mevalonate. The first mechanism might be the creation of cholesterol deficit which could disrupt the integrity of the plasma membrane causing a breakdown of its selective permeability. This could lead to a massive influx of Ca^{++} resulting in a stimulation of the calcium dependent endonuclease. Such a mechanism would be particularly important in those cells that like CEM-C7, which depend on endogenous synthesis to satisfy their cholesterol requirements. The alternate mechanism by which more subtle and, perhaps, more specific alterations in membrane permeability would cause the apoptotic cell damage could involve an accumulation of dolichols either in specific organelles or in selected domains of specific membranes.

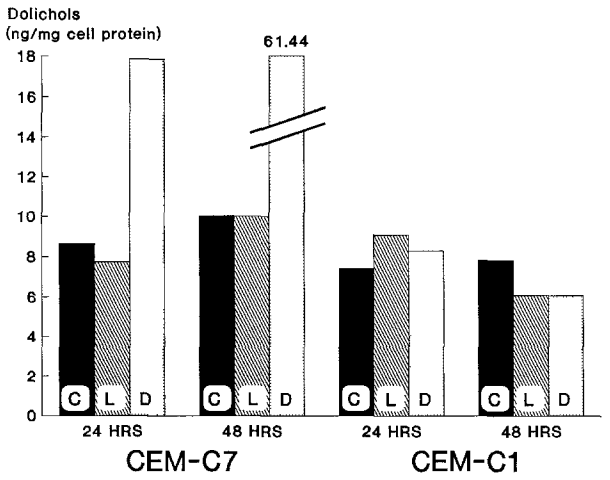

Figure 2. Comparison of the effects of dexamethasone ($1x10^{-7}$ M) and lovastatin (2 μg/ml) on the dolichol content of CEM-C1 and CEM-C7 cells. C = control, D = dexamethasone, L = lovastatin. The values are means of two determinations.

In contrast to dolichol phosphates whose role in asparagin-linked protein glycosylation is well known (14), the function of free dolichols and their acyl esters is poorly understood. Among the few observations is the report by Wong et al. (15) on the localization of dolichols in lysosomes and the more recent preliminary paper by Appelkvist (16) indicating that peroxisomes might be the site of dolichol synthesis. Such information, when considered in context of the extensive studies by Dallner's group in model systems demonstrating changes in model membranes by dolichols (17) and the recent report by Monti et al. (18) showing dolichol-induced changes in permeability to Ca^{2+} of membranes containing phosphatidylcholine and phosphatidylethanolamine, underscore the potential importance of dolichols as regulatory molecules. Studies have been extended recently to the natural membranes by Schroeder et al. (13) who demonstrated fluidity changes in the synaptic plasma membranes after addition of dolichols. The present report raises the possibility that dolichols might be involved in the apoptotic (programmed) cell killing which functions in such biological phenomena as cell replacement, involution of hyperplasia, hormone induced atrophy and regression of tumors. Dolichols seem to be likely as such regulators since they are derived from endogenous synthesis and have a very low rate of turnover. Thus it is possible that their accumulation to a specific threshold level could set in motion the apoptotic cascade.

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