Inhibition of Phosphatidylcholine Biosynthesis following Induction of Apoptosis in HL-60 Cells*

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Induction of apoptosis in HL-60 cells, using a variety of cytotoxic drugs, resulted, in all cases, in inhibition of CDP-choline:1,2-diacylglycerol choline phosphotransferase, leading to an accumulation of its substrate, CDP-choline, and inhibition of phosphatidylcholine biosynthesis. Incubation of the cells with phosphatidylcholine reduced the number displaying an apoptotic morphology following drug treatment, and this was inversely related to the degree to which the drugs inhibited phosphatidylcholine biosynthesis. Inhibition of choline phosphotransferase by two of the drugs, farnesol and chelerythrine, was shown to be due to direct inhibition of the enzyme, while inhibition by the other drugs, etoposide and camptothecin, could be explained by the intracellular acidification that followed induction of apoptosis.

Apoptosis, or programmed cell death, is a process by which organisms eliminate unwanted cells (1, 2). Execution of the apoptotic program leads to a morphologically distinct form of cell death characterized typically by cell shrinkage, nuclear condensation, DNA fragmentation, and the formation of apoptotic bodies. The latter can be phagocytosed in vivo in a process that does not incite the inflammation that normally accompanies cellular necrosis (3). Apoptosis occurs in tissues during development, as a result of normal cell turnover and in the removal of damaged cells. Deregulation of the process has been implicated in the pathogenesis of a variety of diseases including cancer and AIDS (4).

The apoptotic program can be initiated by a variety of stimuli, for example by binding of tumor necrosis factor to its receptor (5) or by withdrawal of factors required for cell proliferation, such as growth factors, cytokines and hormones (6). In addition the process can be induced by a wide range of cytotoxic drugs, many of which are used in cancer therapy (8). We show here that the drugs used previously to induce apoptosis in HL-60 cells (8), and which caused an accumulation of CDP-choline, do indeed inhibit CPT and, as a result, PtdCho biosynthesis. Inhibition of CPT by two of the drugs, farnesol and chelerythrine, could be explained by their direct inhibition of the enzyme. Inhibition of CPT by farnesol has been observed previously (14) and was shown recently to be due to binding of the drug to the enzyme (15). Inhibition of the enzyme by chelerythrine, however, was unexpected, since the drug has been widely used as a potent and selective inhibitor of protein kinase C (PKC). A recent study, however, failed to observe inhibition of PCK by chelerythrine and suggested that the reported effects of the drug should be considered as being independent of PCK inhibition (16). The results presented here suggest that at least some of the biological effects of chelerythrine might be explained by its inhibition of CPT and consequent inhibition of PtdCho biosynthesis. Inhibition of CPT by the other two drugs, etoposide and camptothecin, is shown to be explainable by the cellular acidification that follows the onset of the apoptotic program. Since acidification is a common feature of the program, we examined the possibility that the resultant inhibition of PtdCho biosynthesis could be a component of the execution phase of the apoptotic program. Consistent with this suggestion was the observation that addition of exogenous PtdCho provided protection, at least partially, against the appearance of the morphological features of apoptosis.

EXPERIMENTAL PROCEDURES

Materials—Ammonia, chloroform, ethanol, formaldehyde, hydrochloric acid, methanol, and liquid scintillation fluid (Hi-Safe) were obtained from Fisher Scientific UK (Loughborough, United Kingdom). [methyl-3H]Choline chloride (specific activity 83 Ci mmol−1) and cytidine 5′-diphosphate [methyl-14C]choline (specific activity 55 mCi mmol−1) were obtained from Amersham Pharmacia Biotech (Little Chalfont, UK). Carboxy-SNARF-1-AM was obtained from Cambridge Biosciences (Cambridge, UK) and bisindolylmaleimide I was from Alexis Corp. (UK) Ltd. (Nottingham, UK). Tissue culture materials, culture medium, and medium supplements were supplied by Life Technologies, Inc. (Paisley, UK). All other chemicals were from Sigma (Poole, UK). Thin-layer plates were obtained from Camlab (Cambridge, UK). All chemicals were

1 The abbreviations used are: PtdCho, phosphatidylcholine; PtdEth, phosphatidylethanolamine; PtdSer, phosphatidylserine; PKC, protein kinase C; CPT, CDP-choline:1,2-diacylglycerol cholinephosphotransferase; CCT, CTP:choline-phosphate cytidylyltransferase; Mes, 2-(N-morpholino)ethanesulfonic acid.

* This work was supported by the European Community Framework IV Program (Biotechnology-950207). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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Printed in U.S.A.

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RESULTS

Apoptosis was induced in HL-60 cells using a variety of cytotoxic agents. Camptothecin and etoposide are inhibitors of DNA topoisomerases I and II, respectively (24, 25), and chelerythrine has been used as a specific inhibitor of PKC (26). Farnesol has been shown to inhibit PtdCho biosynthesis, by decreasing CPT activity (14). A more recent study showed that the drug is an inhibitor of the enzyme and that drug binding is competitive with diacglycerol (15).

Induction of Apoptosis Inhibits PtdCho Biosynthesis—

Incubation of HL-60 cells with farnesol, chelerythrine, camptothecin, and etoposide was shown previously, by 31P NMR, to result in the accumulation of CDP-choline (8), implying that the induction of apoptosis by these compounds had resulted in inhibition of PtdCho biosynthesis. PtdCho biosynthesis was investigated in this study by measuring the incorporation of label from [methyl-3H]choline into the water-soluble intermediates of the CDP-choline pathway and into the phospholipid fraction. Chromatographic separation of the phospholipids showed that the majority of the label was incorporated into PtdCho (data not shown).

Farnesol inhibited label incorporation into PtdCho (Fig. 1), increased labeling of CDP-choline and the cellular uptake of choline, and had little effect on labeling of phosphocholine, when compared with control cells (Fig. 2). These results are similar to those found previously in other cell lines and are consistent with inhibition of PtdCho biosynthesis via inhibition of CPT (14, 15). Camptothecin produced a less marked but nevertheless significant inhibition of label incorporation into PtdCho, increased labeling of CDP-choline and uptake of choline, and decreased labeling of phosphocholine. These data are again consistent with inhibition of phosphatidylcholine biosynthesis via inhibition of CPT and with previous 31P NMR data showing an increase in CDP-choline concentration and decrease in phosphocholine concentration following treatment with this drug (8). The temporal relationship between CDP-choline formation and the induction of apoptosis by camptothecin was further investigated by prelabeling the cells for 24 h with [methyl-3H]choline prior to drug treatment. The increase in CDP-choline concentration (Fig. 3A) coincided with an increase in the number of cells displaying an apoptotic morphology, as determined from fluorescence microscopy measurements of nuclear condensation (Fig. 3B). Etoposide produced a much smaller decrease in PtdCho labeling, but this was significantly less than in control cells after 6 h of drug treatment (Fig. 1). There was a significant increase in CDP-choline labeling and cellular uptake of labeled choline but no change in the labeling of phosphocholine (Fig. 2). In conjunction with the earlier 31P NMR data (8), which showed an increase in the concentration of CDP-choline following drug treatment, these results again indicate inhibition of PtdCho biosynthesis via inhibition of CPT. Chelerythrine, unlike the other drugs, markedly reduced the cellular uptake of labeled choline. However, despite this decrease there was a marked increase in label incorporation into CDP-choline, compared with controls, which was consistent with the increased CDP-choline concentration observed previously by 31P NMR (8) and indicates again that there is inhibition of phosphatidylcholine biosynthesis via inhibition of CPT. The decreased labeling of phosphocholine can be explained by the reduced cellular uptake of label and by a decrease in phosphocholine biosynthesis, as the earlier 31P NMR study showed a decrease in the concentration of this metabolite following treatment with this drug.

Effect of the Drugs on Cholinephosphotransferase Activity—

Farnesol was shown previously to inhibit CPT activity in a reagent grade or better.

Cell Culture—HL-60 cells were maintained in RPMI 1640 medium containing 2 g/l-
1 glucose supplemented with 10% fetal calf serum, 2 mM glutamine, 100 unit/ml penicillin, and 100 μg/ml streptomycin.

The cells were grown in spinner flasks (Techne, Cambridge, MA) with 15 μl of 5% CO2/95% air. They were then homogenized at 37 °C in a modified Tris/HCl buffer containing 5 mM cytidine 5-diphosphocholine (50 μM) and PBS (pH 7.4, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 5 mM dye) was then added and the cells harvested, at 37 °C, and resuspended in fresh HEPES buffer (pH 7.4, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 5 mM dye) and resuspended at a density of 1×10^6 cells/ml. This was then incubated with 15 μM camptothecin, 40 μM etoposide, or 60 μM farnesol for a further 30 min at 37 °C and then resuspended in fresh HEPES buffer (pH 7.4). The positions of the debris were removed by centrifugation and the supernatant centrifuged for 24 h in T flasks. They were then harvested by centrifugation and resuspended at a density of 3×10^6 cells/ml in RPMI 1640 medium and incubated for 24 h in T flasks. They were then harvested by centrifugation and resuspended at a density of 1×10^6 cells/ml in RPMI 1640 medium supplemented with [methyl-3H]choline chloride (2 μCi/ml). An apoptosis-inducing agent was added and the cells harvested, by fluorescence microscopy. Cells were washed twice in phosphate-buffered saline, and fixed in 1% formaldehyde (in phosphate-buffered saline) prior to staining with acridine orange at a final concentration of 5 μg/ml and counted on a hemocytometer slide.

Radioisotope Labeling Studies—Cells from spinner flasks were seeded at a density of 1×10^6 cells/ml in RPMI 1640 medium and incubated for 24 h in T flasks. They were then harvested by centrifugation and resuspended at a density of 1×10^6 cells/ml in RPMI 1640 medium supplemented with [methyl-3H]choline chloride (2 μCi/ml). An apoptosis-inducing agent was added and the cells harvested, at various time intervals, by centrifugation following by two washes in ice-cold phosphate-buffered saline. Cellular lipids were separated from aqueous metabolites using a modified Bligh-Dyer procedure (18). The radioactivity associated with the lipid fraction was measured over a 5-min period in a Beckman LS-2800 liquid scintillation counter. Water-soluble choline metabolites were separated by chromatography on silica gel G-coated thin-layer plates using the solvent H2O/ethanol/NH3 (95:48:7, v/v/v), essentially as described in Ref. 19. The positions of the labeled metabolites on the plates were determined by adding an excess of the unlabeled compounds to the extract. CDP-choline was visualized by illumination with uv light (254 nm), with the remaining PtdCho precursors revealed following their exposure to iodine vapor. Approximate RF values in this system were CDP-choline, 0.7; phosphocholine, 0.6; and choline, 0.1. Sections of silica gel containing the choline metabolites were wettet in 0.5 ml of 0.5 m hydrochloric acid and scraped into scintillation vials. The radioactivity released into the scintillant after 24 h was determined as described above.

Flow Cytometric Analysis of Intracellular Acidification—Samples of approximately 6×10^6 cells were removed from incubations with the apoptosis-inducing agents, centrifuged, and resuspended in RPMI 1640 medium supplemented with 50 mM HEPES buffer, pH 7.5. The cells were washed three times with Ca2+-- and Mg2+-free Earle's salts and then resuspended in 10 mM Tris/HCl, pH 7.4, containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 5 μM pepstatin A. After homogenization in a Dounce homogenizer, cell debris was removed by centrifugation and the supernatant centrifuged for 90 min at 100,000× g. The pellet, containing the microsomal fraction, was resuspended in 10 mM Tris/HCl, pH 7.4, and stored on ice prior to enzyme assay.

The assay system contained 175 mM Tris/HCl, pH 8.5, 8 mM MgCl2, 0.5 mM EDTA, 1 mM bovine serum albumin, 2 mM diacglycerol, 0.1 mM cytidine 5-diphospho[methyl-14C]choline and up to 15 μg of protein in a final volume of 140 μl. The reaction was initiated by the addition of the radioactive choline and was allowed to proceed for up to 10 min at 37 °C and terminated by the addition of an equal volume of chloroform/methanol (1:1, v/v). Radioactivity in the chloroform/methanol phase was measured by scintillation counting. Incorporation of label into the phospholipid fraction was shown to be linear with time under these conditions.

Statistical Analysis—Statistical analysis was performed, where appropriate, using Student's t test for unpaired samples.

Effect of the Drugs on Cholinephosphotransferase Activity—

Farnesol was shown previously to inhibit CPT activity in a...
human leukemic cell line, CEM-C1, and more recently to inhibit the activity of the enzyme directly in a microsomal preparation from human lung adenocarcinoma A549 cells (14, 15). This inhibition was competitive with diacylglycerol. We also observed inhibition of CPT activity when farnesol or chelerythrine were added to a microsomal preparation of HL-60 cells (Table I), although the inhibition by farnesol was greater than that observed previously. The inhibition by chelerythrine increased with drug concentration and with the time of preincubation of the drug with the microsomal fraction. However the inhibition appeared not to be due to an indirect effect of PKC inhibition as none was observed with bisindolylmaleimide I, which is a potent and selective inhibitor of PKC (27). Camptothecin and etoposide had no inhibitory effect on the activity of the enzyme. Therefore direct inhibition of CPT activity could explain the accumulation of CDP-choline and inhibition of Pt-dCho biosynthesis observed here with farnesol and chelerythrine but not that observed with camptothecin and etoposide.

Effect of Intracellular pH on PtdCho Biosynthesis—Intracellular acidification is a relatively early and common feature of the apoptotic program (28–32). Since CPT has an alkaline pH optimum (33), this suggested a possible mechanism whereby camptothecin and etoposide might inhibit PtdCho biosynthesis. The number of acidic cells, following the induction of apoptosis, was determined by loading the cells subsequently with the pH-sensitive dye carboxy-SNARF-1-AM. The intracellular...
pH in individual cells was determined using ratiometric flow cytometry. Intracellular pH was assessed by excitation at 488 nm, and the fluorescence emission was monitored at 575 nm and 635 nm (20). In Fig. 4 the intracellular pH for each individual cell is expressed as the ratio of 575:635 nm fluorescence. Untreated cell populations exhibited a relatively narrow range of ratios indicating a homogeneous population of cells with respect to internal pH (Fig. 4A). This peak shifted to a lower ratio, indicating cellular acidification, following treatment with an apoptosis-inducing agent (Fig. 4B). The development of this acidic cell population was time-dependent and preceded the appearance of the morphological features of apoptosis (Figs. 1 and 3), the inhibition of PtdCho biosynthesis (Fig. 1) and the accumulation of CDP-choline (Fig. 2).

Addition of Exogenous Phosphatidylcholine Protects against Apoptosis—Selective inhibition of PtdCho biosynthesis has been shown to induce apoptosis (12, 13) and the addition of exogenous PtdCho to have a protective effect. Induction of apoptosis by farnesol, for example, was shown to be inhibited by the addition of PtdCho to the culture medium (35). This

**TABLE I**

| Compound          | Concentration | Preincubation time of drug with microsomal preparation | CPT activity |
|------------------|---------------|--------------------------------------------------------|--------------|
| Camptothecin     | 15 µM         | 30 min                                                 | 122%         |
| Etoposide        | 40 µM         | 30 min                                                 | 118%         |
| Farnesol         | 10 µM         | 0 min                                                  | 90.8%        |
| Chelerythrine    | 10 µM         | 10 min                                                 | 75.9%        |
|                  | 35 µM         | 30 min                                                 | 33.8%        |
| Bisindolylmaleimide I | 0.5 µM | 0 min                                                  | 97.0%        |
|                  | 0.5 µM        | 10 min                                                 | 94.2%        |

**FIG. 4.** Flow cytometric analysis of cellular acidification following the induction of apoptosis. The histograms show cell count versus the mean fluorescence ratio (at emission wavelengths of 635 nm and 575 nm) for control (A) and farnesol-treated (B) cell populations. C, percentage of acidified cells following treatment with farnesol (●), camptothecin (▪), and etoposide (○). Less than 5% of control cells showed intracellular acidification.

**FIG. 3.** Effect of camptothecin on labeling of the PtdCho precursor CDP-choline. A, cells were preincubated for 24 h with 1 µCi/ml [methyl-3H]choline and then further incubated in the presence (●) or absence (□) of camptothecin for 3 h, after which time the water-soluble PtdCho precursors were separated and analyzed as described under "Experimental Procedures." The radioactivity incorporated into CDP-choline is expressed as disintegrations/min/10⁶ cells. The values given are the means and their S.E. values and represent the results of three independent experiments. Asterisks indicate significantly different from controls at p < 0.05 (*). B, percentage of cells displaying an apoptotic morphology, as determined by fluorescence microscopy (see "Experimental Procedures"). Less than 10% of control cells showed chromatin condensation.
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Inhibition of CPT by farnesol and chelerythrine can be explained by their direct inhibition of the enzyme. Addition of these compounds to an in vitro CPT assay, containing the microsomal fraction from untreated cells, resulted in significant inhibition of CPT activity (Table I). The results obtained with farnesol confirm the results of an earlier study (15); however, the inhibition observed with chelerythrine was unexpected. Chelerythrine, which has been widely used as a selective PKC inhibitor, has been used previously to investigate the role of this protein kinase in apoptosis (26, 38, 39). Since inhibition of CPT and PtdCho biosynthesis are sufficient, on their own, to induce apoptosis, our results suggested that the pro-apoptotic effects of this drug could also be mediated, at least in part, via inhibition of CPT. A recent study has provided evidence that PKC is not inhibited by chelerythrine and that the biological effects of the drug must be mediated via PKC-independent mechanisms (16). Our data support this conclusion. The inhibition of CPT activity appears not to be due to an indirect effect of PKC inhibition, since no CPT inhibition was observed with farnesol or chelerythrine, which has been widely used as a selective PKC inhibitor, inhibited PtdCho biosynthesis. This protection was also observed in this study, where the addition of 100 μM PtdCho to the medium produced an 80% decrease in the number of cells displaying an apoptotic morphology following treatment with this drug (Table II). This protection was also observed with the other drugs used in this study, the degree of protection showing an inverse correlation, albeit a loose one, with the extent to which the drugs inhibited PtdCho biosynthesis. Thus the protection was greater with cells treated with farnesol or chelerythrine, which produced the greatest inhibition of PtdCho biosynthesis, than with cells treated with camptothecin or etoposide, where the inhibition of PtdCho synthesis was more modest (Table II). Furthermore, this protection was specific for PtdCho, as was observed previously (35), and was not observed with phosphatidylethanolamine, cardiolipin, or phosphatidylserine.

**DISCUSSION**

PtdCho is the principal membrane phospholipid in eukaryotic cells, the majority of which is synthesized de novo via the CDP-choline or Kennedy pathway (9). Inhibition of PtdCho biosynthesis has been shown to be sufficient, on its own, to induce apoptosis. Inhibition of CTP-choline-phosphate cytidylyltransferase (CCT; EC 2.7.7.15), which catalyzes the penultimate step in the pathway and is generally considered to be rate-determining for PtdCho biosynthesis (9), was shown to induce apoptosis in a number of cells (13, 36, 37). In Chinese hamster ovary cells, inactivation of a temperature-sensitive mutant of the enzyme resulted in inhibition of PtdCho biosynthesis and induction of apoptosis (12).

Induction of apoptosis, with the cytotoxic drugs used in this study, had been shown previously to interfere with PtdCho biosynthesis (8). Two of the drugs, farnesol and chelerythrine, had already been shown to affect PtdCho biosynthesis. Farnesol inhibited CPT activity in a human leukaemic cell line, CEM-C1, resulting in inhibition of PtdCho biosynthesis and accumulation of CDP-choline (14). A more recent study showed that the drug inhibited the enzyme directly and that this inhibition was competitive with diacylglycerol (15). Chelerythrine, which is regarded as a specific PKC inhibitor, inhibited PtdCho biosynthesis in U937 monocyte-like cells (22). Enzyme assays on cell homogenates showed that there was a reduction in total CCT activity. Camptothecin and etoposide, however, which are inhibitors of topoisomerases I and II, respectively (24, 25), have no obvious connection with phospholipid metabolism.

We have shown in this study that induction of apoptosis in HL-60 cells with these cytotoxic drugs resulted in all cases in an inhibition of extracellular [methyl-3H]choline incorporation into cellular PtdCho (Fig. 1). This inhibition of PtdCho biosynthesis was accompanied by increased label incorporation into CDP-choline (Fig. 2), indicating that the synthesis of this phospholipid was reduced through inhibition of CPT, the enzyme that catalyzes the final step in the Kennedy pathway. The increased labeling of CDP-choline and phospholipid was consistent with our earlier 31P NMR observations, which showed increased CDP-choline concentrations in HL-60 and Chinese hamster ovary cells induced under apoptosis following treatment with these agents (8).

Inhibition of CPT by farnesol and chelerythrine can be explained by their direct inhibition of the enzyme. Addition of these compounds to an in vitro CPT assay, containing the microsomal fraction from untreated cells, resulted in significant inhibition of CPT activity (Table I). The results obtained with farnesol confirm the results of an earlier study (15); however, the inhibition observed with chelerythrine was unexpected. Chelerythrine, which has been widely used as a selective PKC inhibitor, has been used previously to investigate the role of this protein kinase in apoptosis (26, 38, 39). Since inhibition of CPT and PtdCho biosynthesis are sufficient, on their own, to induce apoptosis, our results suggested that the pro-apoptotic effects of this drug could also be mediated, at least in part, via inhibition of CPT. A recent study has provided evidence that PKC is not inhibited by chelerythrine and that the biological effects of the drug must be mediated via PKC-independent mechanisms (16). Our data support this conclusion. The inhibition of CPT activity appears not to be due to an indirect effect of PKC inhibition, since no CPT inhibition was observed with bisindolylmaleimide I, which is a potent and selective inhibitor of PKC (27). The increased CPT inhibition observed following preincubation of the microsomal fraction with chelerythrine may reflect the time required for reaction of the iminium bond in the drug with thiol groups in CPT (16). Addition of camptothecin or etoposide to the CPT assay, however, had no inhibitory effects.

CPT has an alkaline pH optimum of 8.0–8.5 (33) and is inhibited by Ca2+ (40, 41). Since cells undergoing apoptosis frequently show elevated Ca2+ levels (42, 43) and also undergo acidification (28–32), this suggested two possible mechanisms for the inhibition of CPT observed following addition of camptothecin or etoposide to cells. Treatment of cells with the Ca2+ ionophore, A23187, produced an approximately 3-fold increase...
Cell cultures were supplemented with ethanolic solutions of the phospholipids (100 μM) prior to administration of each of the apoptosis-inducing agents. Control cells received ethanol at 0.1% v/v of the culture medium. The values given are the means and their S.E. and represent the results of three independent experiments. Apoptotic morphology was scored as described under “Experimental Procedures.” Less than 10% of controls showed chromatin condensation.

| Treatment                  | Cells displaying an apoptotic morphology (4-h incubation) | Cells displaying an apoptotic morphology (6-h incubation) |
|----------------------------|----------------------------------------------------------|---------------------------------------------------------|
| Camptothecin               | 55.8 ± 3.0                                               | 62.6 ± 2.5                                              |
| Camptothecin + PtdCho      | 38.9 ± 0.6                                              | 40.2 ± 1.9                                              |
| Camptothecin + PtdEth      | 53.4 ± 2.9                                               | 62.2 ± 2.6                                              |
| Camptothecin + cardiolipin | 50.9 ± 2.1                                               | 67.3 ± 0.3                                              |
| Camptothecin + PtdSer      | 52.1 ± 2.0                                               | 63.4 ± 0.6                                              |
| Etoposide                  | 55.7 ± 3.2                                               | 60.1 ± 5.3                                              |
| Etoposide + PtdCho         | 49.9 ± 2.8                                               | 41.6 ± 3.8                                              |
| Etoposide + PtdEth         | 51.7 ± 2.3                                               | 63.9 ± 0.3                                              |
| Etoposide + cardiolipin    | 50.7 ± 1.5                                               | 58.7 ± 1.1                                              |
| Etoposide + PtdSer         | 52.4 ± 2.0                                               | 64.3 ± 2.6                                              |
| Farnesol                   | 51.1 ± 0.1                                               | 67.6 ± 2.6                                              |
| Farnesol + PtdCho          | 14.3 ± 3.3                                               | 17.5 ± 3.5                                              |
| Farnesol + PtdEth          | 48.5 ± 1.0                                               | 67.8 ± 0.4                                              |
| Farnesol + cardiolipin     | 45.0 ± 0.4                                               | 59.3 ± 2.5                                              |
| Farnesol + PtdSer          | 46.5 ± 1.4                                               | 64.0 ± 1.8                                              |
| Chelerythrine              | 45.5 ± 2.4                                               | 63.3 ± 0.7                                              |
| Chelerythrine + PtdCho     | 22.7 ± 3.3                                               | 26.7 ± 1.2                                              |
| Chelerythrine + PtdEth     | 45.1 ± 2.2                                               | 66.8 ± 0.8                                              |
| Chelerythrine + cardiolipin| 45.8 ± 1.8                                               | 63.3 ± 1.4                                              |
| Chelerythrine + PtdSer     | 45.1 ± 0.8                                               | 64.3 ± 3.3                                              |

* Significantly different from cells receiving the agent alone at p < 0.05.

The observation that cellular acidification inhibits PtdCho biosynthesis, coupled with earlier work by others (12, 13) showing that selective inhibition of PtdCho biosynthesis could cause apoptosis, suggested to us that inhibition of PtdCho biosynthesis could be a component of the execution phase of the apoptotic program. Consistent with this proposal is the observation that addition of PtdCho to the medium could protect cells, albeit partially, from induction of apoptosis by camptothecin and etoposide (Table II). Thus addition of PtdCho reduced by 30% the number of cells displaying an apoptotic morphology 4 h after treatment with camptothecin. This protection was specific for PtdCho and was not observed with phosphatidylethanolamine, cardiolipin, or phosphatidylserine. This protection was much greater in cells treated with farnesol or chelerythrine, which we have shown inhibit CPT directly (Table I) and which produce a much greater inhibition of PtdCho biosynthesis (Fig. 1). This protective effect of PtdCho has been observed previously. For example the growth inhibition observed in human CEM-C1 cells, following addition of low concentrations of far-
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nsoles (52) and the apoptotic morphology observed at higher concentrations, were partly abolished by supplementation of the medium with PtdCho (35). Although the rate of PtdCho uptake was only approximately 2% of total cellular PtdCho per hour this was, nevertheless, sufficient to compensate for the defect in PtdCho synthesis caused by farnesol treatment. As was observed here, several other phospholipids were shown to be ineffective. Similar protection has been observed by adding lysophosphatidylcholine to cells incubated with agents that are believed to inhibit PtdCho biosynthesis primarily through their effect on CCT, such as hexadecylphosphocholine or the ether lipid 1-O-octadecyl-2-0-methyl-rac-glycero-3-phosphocholine (37, 53). The protective effect of adding exogenous PtdCho to the medium observed in this study may not only be because it bypasses the block in PtdCho biosynthesis. CPT is activated by PtdCho (41), suggesting that the protection from apoptosis observed in this study could also be due to a stimulation of CPT activity. The mechanism by which inhibition of PtdCho biosynthesis leads to cell death by apoptosis is not known. However a recent study has shown that inhibition of PtdCho biosynthesis results in elevated levels of ceramide (54), which is known to be a potent inducer of apoptosis (55).

In summary we have shown that drug-induced apoptosis in HL-60 cells is accompanied by inhibition of PtdCho biosynthesis. This inhibition was due, in the cases of farnesol and cerelosferine, to direct inhibition of CPT. With camptothecin and etoposide-induced apoptosis the inhibition of PtdCho biosynthesis could be explained by the cellular acidification that accompanied the onset of the apoptotic program, which also inhibits CPT activity. Since cellular acidification is a common feature of apoptosis, this suggests that inhibition of CPT and the resulting accumulation of CDP-choline and inhibition of PtdCho biosynthesis may also be a feature of the process in some cell types.

Acknowledgments—We thank Drs. R. P. Singh and M. Al-Rubeai for help with the flow cytometry experiments, which were conducted in the Department of Chemical Engineering, University of Birmingham, Birmingham, United Kingdom.

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J. Biol. Chem. 1999, 274:19686-19692.
doi: 10.1074/jbc.274.28.19686

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