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Margarete Goppelt-Struebe · Isabel Winter

Effects of hexadecylphosphocholine on fatty acid metabolism: relation to cytotoxicity

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Abstract The cytotoxicity of the antineoplastic drug hexadecylphosphocholine (HePC) was determined in a human monocytic tumor cell line, THP1, and in primary cultures of rat mesangial cells. Both cell types were susceptible to HePC toxicity, the concentrations producing 50% inhibition of cell viability (LD_{50} values) being 7 μg/ml for THP1 cells and 19 μg/ml for mesangial cells. The degree of toxicity was dependent on the culture conditions. In the absence of serum, HePC was highly toxic independent of cell proliferation. As a potential molecular mechanism, the effect of HePC on long-chain fatty acyl metabolism was investigated. HePC had no effect on fatty acid incorporation into cellular lipids or on release of fatty acids from lipid stores. The distribution of labeled fatty acids, however, was shifted from the phospholipid fraction to the triacylglycerol fraction. This effect was in accordance with an inhibition of the activity of the reacylating enzyme lysophosphatide acyltransferase. There was, however, no correlation between the interference with fatty acid distribution and HePC cytotoxicity in vitro. The data argue against interference with membrane fatty acid metabolism as a necessary prerequisite of HePC toxicity, the mechanism of which remains to be elucidated.

Key words. HePC · Lipid metabolism · Cytotoxicity

Introduction

The alkylphosphocholine hexadecylphosphocholine (HePC) is therapeutically used as an antineoplastic drug in the local treatment of skin metastases in breast cancer patients or as an oral formulation for patients with metastatic solid tumors [25, 26]. In vitro, HePC induces morphological changes [14] or cytotoxic effects [3, 24], depending on the cells investigated, and also inhibits the invasion of various tumor cells [20]. The molecular mechanisms by which this and other ether lipid analogues exert their antitumor effects are far from being understood, although multiple in vitro studies indicate interaction of HePC with cellular metabolism.

Due to its lipid structure, effects of HePC on membrane-bound enzymes have been observed. Protein kinase C activity was found to be inhibited by HePC in HL 60 monoblastoid tumor cells [21, 23] and in the epithelial cell line MDCK [4]. Inhibitory effects were also described on Na, K-adenosine triphosphatase (ATPase) [28] and phospholipase C [23]. Translocation of one of the key enzymes in phosphatidylcholine biosynthesis, the cytidine triphosphate (CTP)-cholinephosphate cytidylyltransferase, was inhibited by HePC, whereas no effect of the analogous compound dodecylphosphocholine was observed [5, 22]. Additional effects on various parameters of fatty acid metabolism were described in different tumor cell lines [1].

Thus far, none of these effects has been shown to be specific for tumor cells or to be a prerequisite for the antineoplastic action of HePC. Therefore, we reinvestigated the effects of HePC on fatty acid metabolism in relation to the cytotoxic effects of HePC in the monocytic tumor cell line THP1. Furthermore, the effect of HePC on nontumorous cells, primary cultures of rat mesangial cells, was compared with its effects on tumor cells.

Materials and methods

Materials

HePC was kindly provided by Asta Medica (Frankfurt/Main, Germany). Radioactive fatty acids [^{3}H]-arachidonic acid

M. Goppelt-Struebe (✉) · Winter
Department of Medicine IV, University of Erlangen-Nuernberg, Loschgestrasse 8 1/2, D-91054 Erlangen, Germany

(218 Ci/mmol), [^{3}H]-oleic acid (10 Ci/mmol), and [^{14}C]-linoleic acid (50 μCi/mmol) were obtained from Amersham. Arachidonic acid was supplied by NuChek Prep (USA).

Cell culture

THP1 cells were grown in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS), 50 U penicillin/ml, 50 μg streptomycin/ml, and 1% L-glutamine. Rat mesangial cells were prepared from rat kidneys as previously described [16] and were used between passages 14 and 25. These cells were kept in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 5 ng insulin/ml and the supplements indicated above. For the experiments, mesangial cells were plated in medium containing 5% FCS and were grown to near confluency. Thymocytes were prepared from calf thymus obtained from the local slaughterhouse as described elsewhere [13].

Determination of HePC cytotoxicity

THP1 cells (1×10^5 cells/ml) or preconfluent mesangial cells were incubated with HePC under conditions as indicated. At the end of the incubation, MTT [3-(4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl-tetrazoliumbromide] was added (0.5 mg/ml). After about 60 min the medium was replaced by ethanol to dissolve the blue formazan crystals. Absorbance was read at 550 nm. Within the concentration range used, there was a linear correlation between cell number and formazan absorbance. THP1 cells were also counted in the presence of the dye trypan blue. Mesangial cell proliferation was determined by [^{3}H]-thymidine incorporation as previously described [8].

Incorporation of labeled fatty acids

Radioactive fatty acids (0.1 μCi/assay point, = 0.5 pmol arachidonic acid or 10 pmol oleic acid) were dissolved in ethanol and added to the cell cultures for the periods indicated. The final concentration of ethanol was 0.5% and did not affect cell viability as determined by trypan blue exclusion. At the end of the incubation period, cells were washed two times with phosphate-buffered saline supplemented with 0.1% BSA (PBS/BSA) to remove adherent fatty acids. The adherent mesangial cells were detached by treatment with trypsin/ethylenediaminetetraacetic acid (EDTA, 0.5%:0.2%). The final cell pellet was resuspended in PBS/BSA and the radioactivity was determined by liquid scintillation counting.

Determination of fatty acid release

THP1 cells were labeled with 0.5 μCi oleic acid (50 pmol) or arachidonic acid (2.5 pmol) per assay point. Cells were incubated in medium without serum for 1 h to allow rapid uptake of the fatty acids and were then further incubated overnight in the presence of 5% serum to support cell viability. At the end of the incubation period, the cells were washed two times with PBS/BSA and resuspended in the same buffer. Cells were further incubated with HePC as indicated in Results. At the end of the incubation period, the supernatant was removed and the cells were washed with PBS/BSA. Radioactivity was determined in both fractions.

Analysis of cellular lipids

Cells were incubated with fatty acids and HePC as detailed in Results. At the end of the incubation period the cells were washed with PBS/BSA. Cellular lipids were extracted as previously described [6]. Phospholipids and neutral lipids were separated by thin-layer chromatography using the solvent system hexane/diethyl ether/formic acid (90/60/4, v/v). Lipids were localized according to known standards. The radioactive spots were cut out and the activity was determined by liquid scintillation counting.

Preparation of microsomal membranes

Membranes from mesangial cells or THP1 cells were obtained by sonication of the cells in 20 m*M* TRIS/HCl (pH 8.0), 10% (w/v) glycerol, 1 m*M* EDTA, 5 m*M* 2-mercaptoethanol, and 0.1 m*M* phenylmethylsulfonylfluoride. Calf thymocytes were disrupted by nitrogen cavitation as described earlier [13]. Microsomal membranes were obtained by differential centrifugation at 6,500 and 100,000 *g*. The protein content was determined by a microtiter Bradford assay using BSA as the standard [18].

Determination of acyl-CoA synthase activity

The activity of acyl-CoA synthase was determined in microsomal membranes of THP1 cells as described by Wilson et al. [27] using CoA-SH and linoleic acid as substrates. The activity of the fatty acid was determined to be about 5 nmol mg protein^{-1} min^{-1}.

Preparation of arachidonyl-CoA

The preparation was performed by a modification of the procedure described by Reitz et al. [19]. In brief, 50 mg (150 μmol) CoA was dissolved in a mixture of 35 ml tetrahydrofuran and 15 ml bidistilled water under nitrogen in a 250-ml three-neck flask equipped with a magnetic stirrer and a pH meter. Arachidonyl chloride (100 mg, 130 μmol) was diluted in freshly distilled tetrahydrofuran and added in small portions. Due to the liberated acid, the pH shifted and had to be titrated continuously to pH 8 by the addition of 1 N NaOH. At the end of the reaction the pH was adjusted to 4 with 10% $HClO_4$. After the addition of 200 μl toluol, the tetrahydrofuran was evaporated under nitrogen. Arachidonyl-CoA was precipitated from the milky solution by the addition of 1.5 ml $HClO_4$. After centrifugation for 10 min at 18,000 *g*, the precipitate was washed two times with diethyl ether, then resuspended in 20 m*M* HEPES (pH 7.0) 140 m*M* KCl and kept batchwise at −80°C with 0.2 mg BHT/ml as an antioxidant for several months. The concentration of arachidonyl-CoA was determined by the optical assay described below using excess enzyme.

Determination of lysophosphatide acyltransferase activity

Lysophosphatide acyltransferase activity was determined by an optical assay based on the reaction of liberated CoA with the chromophore 5, 5′-dithio-bis (2-nitrobenzoic acid) (DTNB). Lysophosphatidylcholine and palmitic acid (if indicated) were evaporated from their organic solvents under nitrogen and suspended in buffer (20 m*M* HEPES (pH 7.0), 130 m*M* KCl) by brief sonication. Then, 30–60 μg protein was mixed with 10 nmol arachidonyl-CoA, DTNB (5 m*M* final concentration) and palmitic acid or HePC (if indicated) in a thermostatted cuvette (37°C). This prerun was registered for 5 min to let the solutions warm up and to detect CoA hydrolase activity. The reaction was started by the addition of 25 nmol lysophosphatidylcholine (final volume, 500 μl). The initial velocity was determined by a linear-fit program provided by Gilford (Corning Laboratory Science Company). The enzyme activity was calculated using the molar absorption coefficient E = 13,600 M^{-1} cm^{-1}.

The specific activity was determined to be about 15 nmol mg protein^{-1} min^{-1} for thymocyte membranes, 25 nmol mg protein^{-1} min^{-1} for membranes of THP1 cells, and 60 nmol mg protein^{-1} min^{-1} for membranes of rat mesangial cells.

Presentation of data

If not indicated otherwise, data represent mean yalues ± SD for n independent experiments. Significance was calculated by Student's t-test.

Results

Cytotoxic effects of HePC

HePC was incubated with THP1 monocytic tumor cells for 48 h and cell viability was assessed by cell counting in the presence of the dye trypan blue. Cell viability decreased with increasing concentrations of HePC (Fig. 1). Corresponding results were also obtained with the MTT test, which is based on the reduction of the tetrazolium salt to blue formazan by living cells (Fig. 1) [2, 17]. Cell viability was reduced to 50% by an HePC concentration of 6.9 ± 2.5 μg/ml ($n = 5$).

Mesangial cells were less sensitive to the cytotoxic effects of HePC. At a concentration of 10 μg/ml, cell viability was not yet affected (106% ± 13.5%, $n = 5$), but it was reduced to 42.4% ± 8.7% ($n = 5$) by incubation with HePC at 20 μg/ml. Half-maximal cytotoxicity was obtained at an HePC concentration of 18.8 ± 2.5 μg/ml ($n = 4$). Proliferation of mesangial cells as determined by thymidine incorporation was not affected by HePC at 10 μg/ml (93.9% ± 9.1%, $n = 4$, as compared with control cells) but was abolished at an HePC concentration of 20 μg/ml (4.4% ± 2.0%, $n = 4$). These data indicate that primary cells in culture are growth-inhibited and killed by HePC within a very narrow concentration range that is not much different from the concentrations effective in tumor cells.

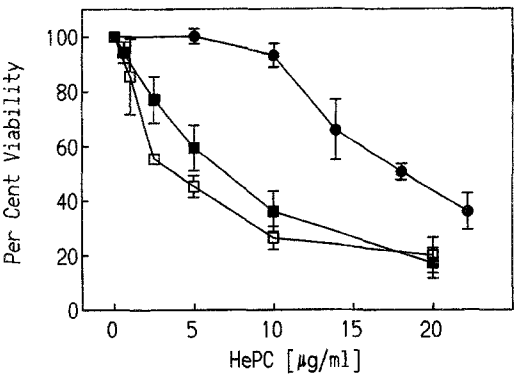

Fig. 1 Cytotoxicity of HePC toward THP1 cells and mesangial cells. THP1 cells (■, □) or proliferating mesangial cells (●) were incubated with HePC in medium with 5%FCS for 48 h. At the end of the incubation period, cell viability was determined by trypan blue exclusion (□) or the MTT test (■). Data represent percentages of viable cells as compared with control cells grown in the absence of HePC, expressed as mean values ± SD for 3 independent experiments

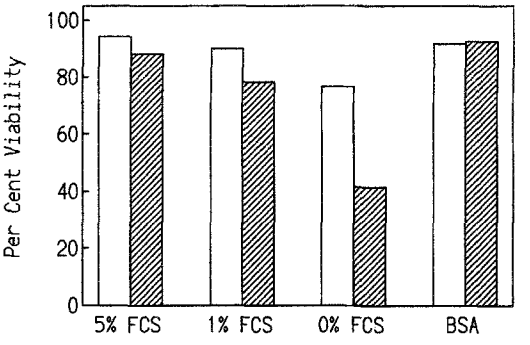

Fig. 2 Effect of culture conditions on HePC cytotoxicity. THP1 cells were incubated with HePC at concentrations of 5 (*open bars*) or 10 (*hatched bars*) μg/ml for 5 h in medium containing 5% FCS, 1% FCS, 0.1% BSA, or no additive. Cell viability was determined by the MTT test. The absorbance of control cells cultured in the same medium in the absence of HePC was set to 100%. Data represent mean values for duplicate determinations in a representative experiment of a series of 3 similar ones

Effect of different culture conditions on HePC cytotoxicity

THP1 cells were incubated with different concentrations of HePC in the presence of 5% or 1% serum or of 0.1% BSA or in medium without additional protein. Cytotoxicity was determined by the MTT assay after 5 h (Fig. 2). The cytotoxicity of HePC markedly increased with decreasing protein concentrations in the medium. The effect occurred very rapidly and was observed after 15–30 min. After this time, about 25% of the cells were dead as determined by counting in the presence of trypan blue, when the cells were incubated with 5 μg HePC/ml in serum without additives. Similar results were obtained with mesangial cells (Fig. 3b).

Incorporation of fatty acids

THP1 cells were incubated for 30 min with HePC at the concentrations indicated in medium containing 5% FCS, 0.1% BSA, or no additive (Fig. 3). The cells were further incubated for 60 min with radioactive [^{3}H]-labeled oleic acid. At the end of the incubation period the amount of fatty acid incorporated was determined. Cell viability was assessed by trypan blue exclusion. Depending on the culture conditions, the amount of fatty acid incorporated varied, being dependent on the fatty acid binding to serum protein or albumin. As long as the cells remained viable, oleic acid incorporation was not affected by HePC (Fig. 3). When the cells were cultured in the absence of protein, oleic acid

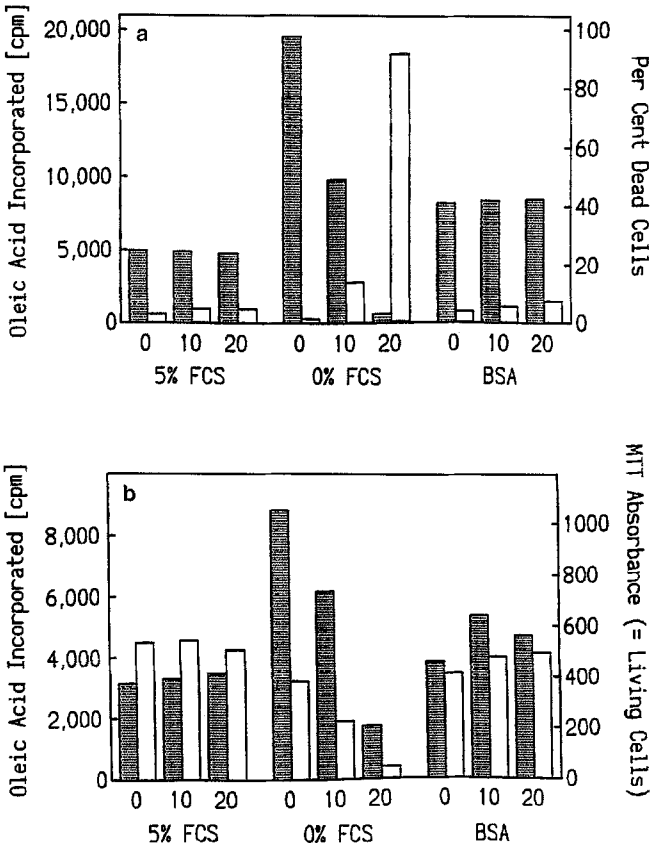

Fig. 3a, b Fatty acid incorporation into cellular lipids. **a** THP1 cells were incubated with HePC at 0, 10, 20 μg/ml for 90 min in medium containing 5% FCS, no FCS, or 0.1% BSA. Oleic acid (0.1 μCi/ml = 10 pmol/ml) was added for the last 60 min. Thereafter, the cells were washed and the cellular content of oleic acid was determined by liquid scintillatation counting (*hatched bars*). Data represent mean values for triplicate determinations in a typical experiment. The percentage of dead cells was determined by trypan blue exclusion (*open bars*). **b** Mesangial cells were incubated with HePC as described in **a**. MTT absorbance was used as a measure of living cells (*open bars*)

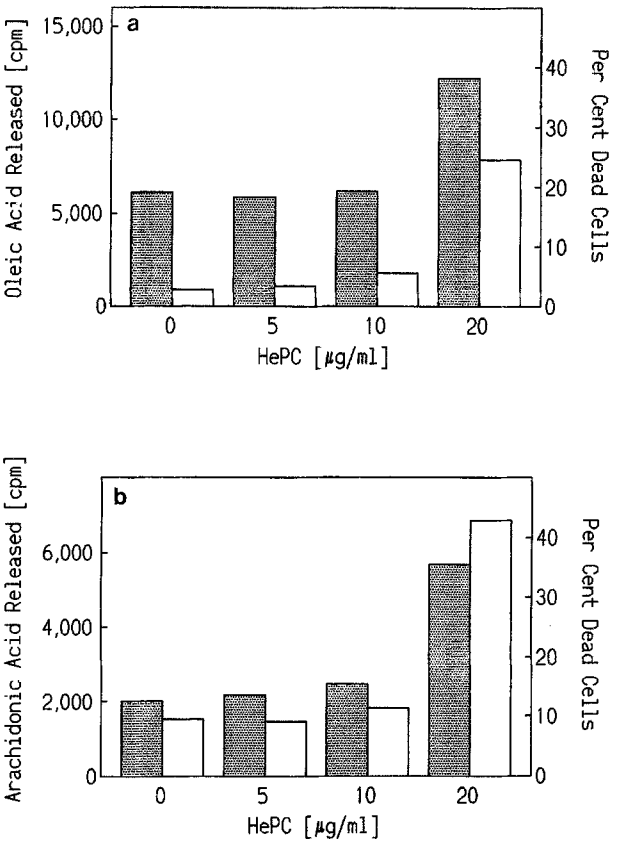

Fig. 4a, b Fatty acid release from prelabeled THP1 cells. THP1 cells were labeled overnight with **a** oleic acid or **b** arachidonic acid. The cells were then treated with HePC as indicated for 2 h in medium with 0.1% BSA. Radioactivity released into the supernatant was determined by liquid scintillation counting (*hatched bars*, means of duplicate determinations). In parallel experiments, cell viability was determined by trypan blue exclusion: the percentage of dead cells is represented by *open bars*

incorporation decreased in parallel with the loss of viability (Fig. 3a). The same results were obtained with mesangial cells, where cytotoxicity was determined by the MTT assay, whereby the absorbance corresponded to the proportion of living cells (Fig. 3b).

Effect of HePC on the release of fatty acids

THP1 cells were labeled overnight with oleic acid. Then the cells were washed with PBS/BSA to remove nonincorporated fatty acid and were further incubated for 2 h with different concentrations of HePC in medium containing 0.1% BSA to trap released oleic acid. In parallel assays, cell viability was determined by counting in the presence of trypan blue. Under these conditions, cell viability was not affected by HePC at concentrations of up to 10 μg/ml (Fig. 4a). The release of oleic acid from these cells was the same as that from control cells (93.5% ± 10.6%, $n = 6$). Doubling of the HePC concentration led to a variable degree of cytotoxicity, which was accompanied by the release of radioactivity into the supernatant (127% ± 41%, $n = 6$). Similar results were obtained when the polyunsaturated fatty acid arachidonic acid was used (Fig. 4b). At a 10-μg/ml concentration of HePC the release was 104% ± 8% ($n = 5$), and at 20 μg/ml the release of arachidonic acid-containing activity was enhanced to 156% ± 49% ($n = 5$), corresponding to the reduction in cell viability.

Effect of HePC on fatty acid distribution

For assessment of the long-term effects of HePC, THP1 cells were incubated overnight with HePC in medium with 5% serum. Thereafter, the cells were washed and incubated with arachidonic acid in medium without

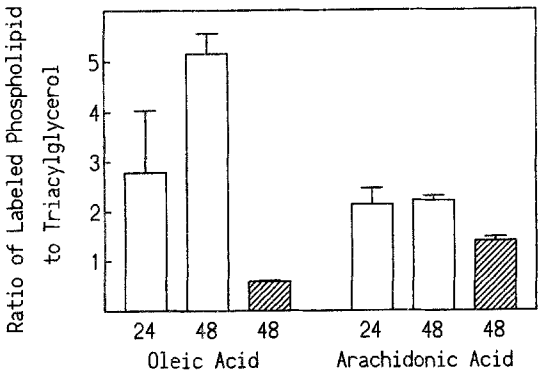

Fig. 5 Effect of HePC on the distribution of fatty acids among lipid classes. THP1 cells were incubated for 24 h with oleic acid or arachidonic acid in medium with 5% FCS. The washed cells were further incubated for 24 h in the presence (*hatched bars*) or absence (*open bars*) of HePC. At the end of the incubation period, the distribution of the labeled fatty acid among phospholipids and triacylglycerols was determined by thin-layer chromatography. The ratio of labeled phospholipid to labeled triacylglycerol for 3 parallel incubations is depicted (mean values ± SEM)

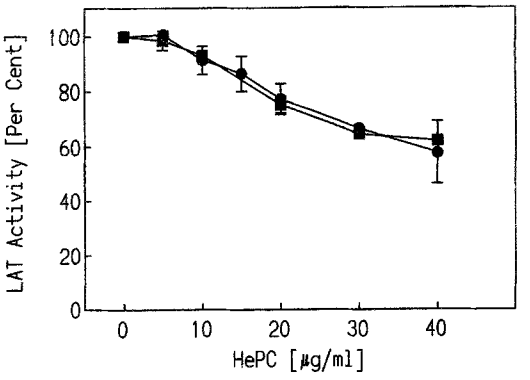

Fig. 6 Inhibition of lysophosphatide acyltransferase activity by HePC. Microsomal membranes were prepared from THP1 cells and mesangial cells as described in Materials and methods. The activity of the lysophosphatide acyltransferase was determined using arachidonyl-CoA and lysophosphatidylcholine as substrates in the presence of various concentrations of HePC. The activity of control cells was set to 100%. Data represent mean values + SD for triplicate determinations of a mesangial cell preparation (●) and for 3 THP1 preparations (■)

serum for 1 h. Control experiments showed that cell viability was not affected by fatty acid incubation under these conditions. The distribution of the fatty acid among cellular lipids was determined by thin-layer chromatography. In control cells, 68.7% ± 10.9% ($n = 3$) radioactivity was found in the phospholipid fraction. The distribution was not significantly affected by incubation of the cells with HePC at 2.5 μg/ml (61.0% ± 7.8% of the arachidonic acid being incorporated into phospholipids, $n = 3$). At a 10 μg/ml concentration of HePC, however, the incorporation into phospholipids was reduced (49.5% ± 5.5%, $n = 3$; $P < 0.01$), with an increasing percentage of activity being found in neutral lipids, primarily triacylglycerols. Similar results were obtained when oleic acid was investigated (data not shown).

The effects of HePC on fatty acid distribution were also investigated in THP1 cells prelabeled with oleic acid or arachidonicy acid. These cells were then incubated with HePC for 24 h in medium with 5% serum. Thereafter, lipids were extracted and the distribution of the labeled fatty acid was investigated by thin-layer chromatography. Under these conditions, the fatty acid was found almost exclusively in the phospholipid fraction, with very little activity remaining within the triacylglycerol fraction. The distribution of arachidonic acid varied among different experiments, although it was stable within one experiment after 24 and 48 h, whereas that of oleic acid was shifted from neutral lipids to phospholipids (Fig. 5). Upon incubation with HePC at 10 μg/ml, a shift of labeled fatty acid from phospholipids to neutral lipids was observed. The ratio of phospholipid to triacylglycerol was 19.5 ± 12.3 in control cells after 48 h and was 4.6 ± 3.7 in HePC-treated cells labeled with arachidonic acid ($n = 5$, $P < 0.05$). In oleic acid-labeled cells, the ratio of phospholipid to triacylglycerol was reduced from 3.5 ± 1.8 in control cells after 48 h to 0.7 ± 0.2 ($n = 2$) in HePC-treated cells.

Effect of HePC on reacylating enzymes

Fatty acid incorporation into phospholipids is dependent on two types of enzymes, the acyl-CoA synthases and the lysophosphatide acyltransferases. The activities of both enzymes were determined in vitro and the effect of HePC on the enzyme activities was determined. Acyl-CoA synthase activity was determined in a microsomal fraction of THP1 cells using linoleic acid and CoA-SH as substrates. HePC concentrations of up to 20 μg/ml had no effect on the in vitro formation of linoleoyl-CoA.

The activity of lysophosphatide acyltransferase was determined in the microsomal fraction of THP1 cells using arachidonyl CoA and lysophosphatidylcholine as substrates. In this assay, HePC inhibited the in vitro enzyme activity in a concentration-dependent manner (Fig. 6). The same inhibition was obtained when membranes from rat mesangial cells (Fig. 6) or calf thymocytes (Figs. 7, 8) were investigated. As we had previously used calf thymocytes to characterize the lysophosphatide acyltransferase [7], the mechanism of enzyme inhibition was investigated in membranes from these cells. HePC was an uncompetitive inhibitor with respect to both of the enzyme substrates (arachidonyl-CoA and lysophosphatidylcholine), reflecting the complex interactions between membrane-bound enzymes and substrates (Figs 7a, 7b). On a molar basis, the enzyme inhibition produced by HePC (20 μg/ml = 50 μ*M*) was comparable with the effect of hexadecanoic acid (palmitic acid; Fig. 8). Incubation of

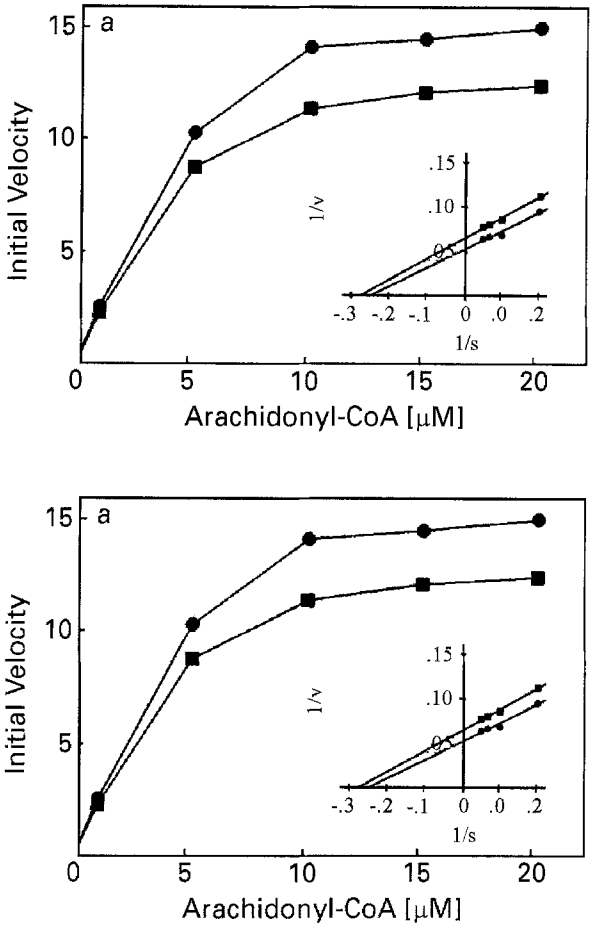

Fig. 7a, b Uncompetitive inhibition of lysophosphatide acyltransferase activity by HePC. Lysophosphatide acyltransferase activity (initial velocity, nmol mg protein^{-1}min^{-1}) was determined in microsomal membranes obtained from calf thymocytes in the presence of varying concentrations of **a** arachidonyl-CoA and **b** lysophosphatidylcholine as substrates and 15 μg HePC/mol (■) as an inhibitor (*Inserts* Data transformed according to Michaelis-Menten)

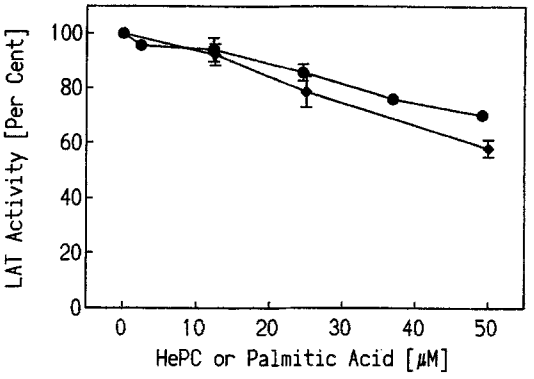

Fig. 8 Inhibition of lysophosphatide acyltransferase activity by HePC or palmitic acid. Lysophosphatide acyltransferase (*LAT*) activity was determined in microsomal membranes from calf thymocytes in the presence of palmitic acid (◆) or HePC (■, 20 μg/ml = 50 μM). Data represent mean values ± SEM for 3 different preparations each

THP1 cells with palmitic acid, however, did not lead to the cytotoxicity observed for HePC under comparable conditions.

Discussion

Different sensitivity to the cytotoxic action of the antineoplastic drug HePC has been described in various cell lines. Thus far, the molecular basis for this variable susceptibility, even among tumor cells, has not become evident [1]. In our studies we compared the effects of HePC on the monocytic tumor cell line THP1 with the effects on primary cultures of rat mesangial cells in an attempt to define the specific antitumor effects of HePC. The susceptibility of THP1 cells was comparable with that of U937 cells, another human monocytic cell line, which has many characteristics in common with THP1 cells. Half-maximal cytotoxicity was reported for HePC concentrations ranging from 7 to 16 μg/ml [1, 24], which is in good agreement with the value of 7 μg/ml obtained in our experiments. Although mesangial cells were less sensitive to HePC concentrations of up to 10 μg/ml, they were susceptible to concentrations ranging between 15 and 20 μg/ml, which were not essentially different from those effective in terms of tumor-cell kill. On administration of HePC to mice, the drug accumulated in the kidney, but no obvious toxic symptom was observed [24]. In humans, however, renal dysfunction was observed in 30% of patients treated orally with HePC for various mesenchymal tumors [26]. Autopsy of one of those patients revealed interruptions of the epithelial lining. However, susceptibility of mesangial cells might also contribute to an impairment of renal function by HePC. Meanwhile, HePC is no longer considered to be viable agent for systemic therapy of tumors because of its gastrointestinal toxicity [10].

The antineoplastic effect of alkyllysophospholipids, especially ET-18-OCH_3, has been related to its interference with long-chain fatty acid metabolism, which is a prerequisite for maintenance of the integrity and functionality of cellular membranes [9]. Therefore, it was tempting to speculate that HePC, which is structurally related to the alkyllysophospholipids, might also interfere with fatty acid incorporation or release. All experiments on lipid metabolism were done in parallel with cytotoxicity evaluations because HePC cytotoxicity proved to be very dependent on the composition of the medium, which had to be modified for optimal investigation of fatty acid incorporation and release. In the absence of serum, HePC was highly toxic within minutes; under these conditions its cytotoxicity was independent of cell proliferation.

HePC had no effect on fatty acid incorporation into whole cells nor did it affect fatty acid liberation.

Different fatty acids were used, because phospholipases with different fatty acid specificities might have been modulated by HePC. Whenever an effect on incorporation or release was detected, it was accompanied by a cytotoxic effect of HePC. A reduced incorporation of fatty acids was thus attributed to a decrease in the number of living cells, and an enhanced release of fatty acids most likely accompanied cellular destruction rather than being the cause of it. When concentrations of HePC were used that led to a considerable loss of viability, after 24 or 48 h, no early change in membrane fatty acid metabolism was observed that might support the concept of membrane damage being the cause of later cell death.

More subtle changes were observed when the fatty acid distribution among different lipid classes was observed. In the presence of HePC, incorporation of exogenous fatty acids was shifted from phospholipids to neutral lipids. Similarly, rearrangement of acyl chains, which continuously occurs in living cells and is mediated by the deacylation-reacylation cycle [11], was impaired and fatty acids were acylated to triacylglycerols. A shift of arachidonic acid incorporation from phospholipids to triacylglyercols due to phospholipase activation was observed in HL60 cells treated with lead compounds and was related to the toxicity of the latter [12, 15]. A direct effect of HePC on phospholipase A_2 activity could not be measured because HePC interfered nonspecifically with the in vitro assay (data not shown). As HePC had no effect on fatty acid liberation, there was no indication for an interference of HePC with phospholipase activity. Therefore, the effects of HePC on the reacylating enzymes acyl-CoA synthase and lysophosphatide acyltransferase were investigated.

The acyl-CoA synthase activity determined in membrane fractions obtained from THP1 cells was not affected by HePC. Lysophosphatide acyltransferase had previously been shown to be a target of alkyllysophocholine action. Inhibition of the enzyme was competitive with respect to the substrate lysophosphocholine and correlated with the sensitivity to alkylphosphocholine cytotoxicity of the cells investigated [9]. However, HePC inhibited the lysophosphatide acyltransferase from all cellular sources investigated to the same extent, even if the cells were rather insensitive to HePC cytotoxicity (e.g., human peripheral blood mononuclear cells; data not shown). Inhibition was of the uncompetitive type with respect to the two substrates arachidonyl-CoA and lysophosphocholine. The effect of HePC was comparable with that of palmitic acid, which also contains a hexadecyl chain. Palmitic acid was nontoxic for the cells, clearly indicating that the inhibition of lysophosphatide acyltransferase cannot be considered to be an essential cause of HePC cytotoxicity.

We could thus exclude that disturbance of the lipid composition of cellular membranes by interference with long-chain fatty acyl metabolism is a prerequisite for HePC toxicity. The molecular mechanism of the antineoplastic activity of HePC remains to be elucidated for a further definition of the beneficial and toxic effects of this compound.

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