Cytotoxic Efficacy and Influence on Cellular Phospholipid Metabolism of 2-Hydroxy- and 2-O-Acetyl-Octadecylphosphocholines

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Abstract. The cytotoxic efficacy and influence on phospholipid composition of the new alkylphosphocholines (APC) 2-hydroxy and 2-O-acetyl-octadecylphosphocholines both synthesised in R- and S-configuration (R/S-OH and R/S-O-acetyl) were examined in vitro using HL-60 and MDA-MB-468 cells. IC50- and LC50-values were measured by MTT- and cell count assay. All tested APC showed higher or similar cytotoxic efficacy compared to the well known APC hexadecylphosphocholine (HePC). However, while S-configured APC (IC50) revealed considerably higher cytotoxic activities, only R-(natural)-configured APC caused significant changes in the phospholipid composition of tumour cells. Further investigations revealed an increase in R-O-acyl and loss of PC up to 70% in the membrane of both cell lines. Similar to PC, R-O-acyl bears two long non-polar hydrocarbon chains and stabilises cell membranes structurally, thus, possibly explaining less cytotoxicity and lack of apoptosis induction by R-configured APC. Nevertheless, an enrichment of R-O-acyl up to 70% at the expense of PC in cell membrane is tremendous and may inhibit tumour development by influencing the intracellular lipid signalling. In conclusion, our findings reveal the antitumoral efficacy of all tested new APC and offer new perspectives in drug development targeting phospholipid metabolism.

Alkylphosphocholines (APC) are synthetic analogues of lysophosphatidylcholine (lyso-PC) and represent a new class of antineoplastic and antiparasitic membrane agents displaying numerous biological properties, including the efficient induction of apoptosis (1-8). Well-known examples of APC are 1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine (Et-18-OCH3) and hexadecylcholine (HePC) (9). HePC is used in topical treatment of skin metastases resulting from breast cancer or lymphomas (10-12). Furthermore, HePC shows a remarkable antiparasitic effect in vitro and in vivo even at low doses (13-15). Unfortunately, the therapeutic index of HePC for anticancer treatment is limited due to its severe side-effects (16).

In contrast to natural lyso-PC, HePC bears no ester or hydroxyl group adjacent to the phosphocholine head group (Table I). Therefore, HePC shows only marginal substrate properties to phospholipids metabolising enzymes resulting in an increased half-life in serum. This allows for accumulation of HePC in the cell membrane and is probably the main reason for its numerous side-effects (17, 18).

In order to develop APC with improved antineoplastic activity and fewer side-effects than those of HePC, a group of analogues were synthesised (Table I). These new APC contain additional functional groups (acetic ester and hydroxyl groups) adjacent to the phosphocholine headgroup (2-position) and have been synthesised in R-(natural)-, as well as in S-(unnatural)-configurations. In order to achieve roughly the same effective length of the apolar hydrocarbon chain as HePC, the new compounds were based on the octadecyl chain instead of the hexadecyl chain (19). As shown by Krug et al., the S- but not the R-configured APC are able to induce apoptosis in leukaemia cells at the level of CD95 (20-22). Due to their
additional functional groups and their similarity to lyso-PC, the new R-(natural) configured APC were expected to show increased interactions with lipid metabolizing enzymes like acylating enzymes or lysophospholipases. The acylation of lyso-PC to PC is described as an important pathway of PC-synthesis, particularly dominant in proliferating cells like cancer cells (23).

The influence of the new, naturally configured APC on lipid metabolism and membrane structure in general, as well as specific influence on acylating enzymes in particular are presumed to cause an improved cytotoxic effect in fast growing cells or in cells with a rapid phospholipid turnover. Moreover, interaction of the new APC with lipid metabolising enzymes may decrease their half-lives, thus, reducing side-effects in vivo. The antitumoral efficacy of the new APC was, thus, studied using the leukaemic cell line HL-60 and the breast cancer cell line MDA-MB-468. Moreover, the influence of the new APC on the phospholipid metabolism of the cell lines was analysed focusing on the acylation of phospholipids. All results were compared to those of the well known HePC.

Materials and Methods

Chemicals. The new APC and HePC were synthesised in accordance with the literature (19, 24). Triethylamine, cupric sulphate, methanol, sodium hydrogen-carbonate, phosphoric acid, chloroform, perchloric acid, dioxin, acetic acid (all reagent grade) and HPTLC plates (silica gel 60 (200x100 mm) were obtained from Merck (Darmstadt, FRG). 14C-labelled-1-O-palmitoyl-sn-3-glycero-phosphatidylcholine (L-lyso-3-phosphatidylcholine, 1-(1-14C) palmitoyl), specific activity 50-62 mCi/mmol) was purchased from Amersham Pharmacia, Biotech Export, England.

Cell lines. HL-60 cells (acute promyelocytic leukaemia) (25) were grown in RPMI-1640 medium, supplemented with 15% foetal calf serum, penicillin (100 U/ml), streptomycin (100 µg/ml), L-glutamin (100 µg/ml) (all Bio Wittaker, Verviers/ Belgium) and sodiumhydrogencarbonate (2 g/l). MDA-MB-468 cells (breast carcinoma cells) (26) were grown in EMEM medium, supplement with 10% foetal calf serum, Penicillin (100 U/ml) and streptomycin (100 µg/ml), L-glutamin (100 µg/ml) (all Bio Wittaker, Verviers/Belgium) and sodiumhydrogencarbonate (2 g/l). Both cell lines were cultured at 37°C, 5% CO₂.

Cytotoxic activities. LC5₀-values: HL-60 and MDA-MB-468 cells were seeded at 1x10⁵ cells/ml in 24-well plates (Greiner, FRG) in a final volume of 1 ml per flat bottom well. After 12 (HL-60 cells) or 24 h (MDA-MB-468 cells) of incubation, various concentrations of APC were added. The cells were incubated for an additional 48 h and counted using a CASY-1-cell counter (Analyser System Model TTC, Schär-fesystem, Reutlingen, FRG).

IC5₀-values: HL-60 and MDA-MB-468 cells were seeded at 1x10⁵ cells/ml in 96-well plates (Greiner, FRG) in a final volume of 100 µl per flat bottom well. After 12 (HL-60 cells) or 24 h (MDA-MB-468 cells) of incubation, various concentrations of APC were added and the cells were incubated for an additional 24 h, followed by the addition of 10 µl of the MTT labelling reagent (MTT colorimetric assay, Boehringer Mannheim, FRG) (27) to each well and incubation for 4 h. Resulting formazan salts were made soluble by adding a solubilisation solution (100 µl/well). After an additional incubation of 12 h, the resulting dye was determined using a microplate reader (Elisa Reader, Modul MR 7000, Dynettech, Iceland; absorption wavelength: 550 nm; reference wavelength: 630 nm).

Influence of the APC on cellular phospholipid composition. HL-60 and MDA-MB-468 cells were seeded at 1x10⁵ cells/ml and incubated for 12 and 24 h, respectively. APC (IC5₀) were added and the cells were incubated for an additional 48 h. The numbers of cells were determined and the cellular lipids were extracted, separated by HPTLC, stained and quantified using a densitometer (TLC Scanner II, Camag, Berlin, FRG) (29). Prior to the lipid extraction, the cells were washed with PBS. Using 1 ml of PBS the cells were transferred into glass vials; 2 ml of chloroform/methanol (2:1, v/v) were added and the mixtures were vortexed for 30 sec. For phase separation, the tubes were centrifuged at 3,000 xg for 5 min. The (lower) chloroform phase was transferred to a second set of test tubes. Extractions were repeated twice using chloroform/methanol (3:1 and 4:1 v/v, respectively). The chloroform phases were combined and the solvents carefully removed using a gentle stream of nitrogen. For HPTLC, the silica gel-plates were pre-run with the mobile phase chloroform/methanol/triethylamine/water (30:35:34:8 v/v/v/v). Samples and calibration solutions were automatically streaked on the HPTLC plate using a Linomat IV (Camag, Berlin, FRG). The samples were dissolved in a volume of 100 µl of chloroform/methanol (2:1 v/v) before being applied as 5 mm lines approximately 15 mm from the lower edge of the plate. The distance between each sample was 10 mm. The plates were developed in glass tanks (height: 8.5 cm). After drying the plates at 180 °C for 10 min on a heating plate (Desaga, Heidelberg, FRG), the lipids were dried with a cupric sulphate solution (10% w/v) in phosphoric acid (8% w/v). For this, the HPTLC plate were soaked in the dye solution for 15 s and subsequently put on a heating plate at 180 °C for 10 min. The
phospholipid spots were quantified using a TLC-SCANNER II (CAMAG, Berlin, FRG) at an absorption wavelength of 420 nm. 

**Kinetics of lyso-PC reacylation.** HL-60 and MDA-MB-468 cells were seeded at 1x10^5 cells/ml and incubated for 12 (HL-60 cells) or 24 h (MDA-MB-468 cells). Fifty µl of 14C-labelled-1-O-palmitoyl-sn-glycero-phosphatidylcholine (0.04 µCi/ml) were added and the cells were again incubated for various periods (3, 6, 12, 24 h). The numbers of cells were determined and the cellular lipids were extracted, separated by HPTLC and stained by cupric sulphate solution, as described above. Quantification of the radiolabelled lipid spots was performed using a phosphoimager (BAS 1000, FUJIX, Japan).

**Influence of the APC on reacylation.** HL-60 and MDA-MB-468 cells were seeded at 1x10^5 cells/ml and incubated for 12 (HL-60 cells) or 24 h (MDA-MB-468 cells). APC (IC_50-) were added together with 50 µl 14C-labelled-1-O-palmitoyl-sn-glycero-phosphatidylcholine (0.04 µCi/ml). The cells were incubated for an additional 10 h (HL-60 cells) or 3 h (MDA-MB-468 cells). Isolation and quantification of the radiolabelled lipid spots were performed as described above.

**Identification of R-O-acyl.** HL-60 cells were seeded at 1x10^5 cells/ml and incubated for 12 h. R-OH (IC_50-) was added and the cells were incubated for an additional 48 h. The cellular lipids were extracted, separated by HPTLC and dyed as described above. The PC and the new lipid spot were both scratched from the glass plate and were incubated with H_2O/perchloric acid/dioxan (1:2:4 v/v/v) for 1 h at 90°C. The solution was then cooled and neutralised using NaOH (6 N, approximately 400 µl). Water (250 µl) was added and the dispersion was extracted with acetic ester (500 µl). The lipids were separated by HPTLC (mobile phase: chloroform/methanol 9:1 v/v) and dyed with cupric sulphate solution as described above.

**Results**

All tested APC showed high cytotoxic activity (LC_50-) and high inhibition of cell metabolism (IC_50-) in both cell lines (Table II). With the exception of R-OH, all APC had similar or lower IC_50- and LC_50-values than HePC, one of the most cytotoxic APC known (9). Comparison of the S-(unnatural)-configured APC with the R-(natural)-configured APC showed lower IC_50- and LC_50-values of the unnatural configured APC (Table II). In addition, the LC_50-values for MDA-MB-468 cells were generally higher than for HL-60 cells. In contrast, APC bearing a hydroxyl group (S-/R-OH) inhibited the metabolism (IC_50-) of MDA-MB-468 cells to a higher degree than the metabolism of HL-60 cells (Table II).

To investigate changes in membrane phospholipid content, the phospholipid composition after incubation of the HL-60 and MDA-MB-468 cells with the new APC (IC_50-) was analysed. As demonstrated in Figures 1 and 2, no (MDA-MB-468 cells) or only little (HL-60 cells) differences in the phospholipid composition between the control cells and those cells incubated with S-configured APC or HePC could be seen. S-O-acetyl and S-OH caused a slight increase in total cell lipid content in HL-60 cells which resulted mainly from an increase of phosphatidylethanolamine (PE) (144%/155% of control), fatty acids (118%/110% of control) and sphingomyelin (SM) (88%/116% of control). No significant alteration of the PC proportion (93%/103% of control) could be observed. In contrast, in the HL-60 cells, HePC induced a slight decrease in the total lipid content which resulted from a decrease in fatty acids (80% of control), PC (90% of control) and SM.

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**Table II. Influence of HePC and the new APC on the metabolism (IC_50-) and death (LC_50-) of HL-60 and MDA-MB-468 cells.**

<table>
<thead>
<tr>
<th>APC</th>
<th>HL-60 cells</th>
<th>MDA-MB 468 cells</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IC_50</td>
<td>LC_50</td>
</tr>
<tr>
<td>HePC</td>
<td>4.8±1.8</td>
<td>20.9±2.2</td>
</tr>
<tr>
<td>R-OH</td>
<td>32.4±6.2</td>
<td>43.1±3.7</td>
</tr>
<tr>
<td>S-OH</td>
<td>3.2±0.1</td>
<td>86.1±4.8</td>
</tr>
<tr>
<td>R-O-acetyl</td>
<td>9.4±0.7</td>
<td>17.2±1.3</td>
</tr>
<tr>
<td>S-O-acetyl</td>
<td>20.3±1.6</td>
<td>69.7±4.3</td>
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</tbody>
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IC50-values were determined using an MTT-assay, LC50-values were determined using a cell count assay (CASY-1-cell counter) after an incubation period of 48 h. Data represent the mean±SD of 3 independent experiments, each done in triplicate.

**Figure 1.** Separation of cellular lipids by HPTLC after incubation of HL-60 and MDA-MB-468 cells with APC (IC50): HL-60 and MDA-MB-468 cells were seeded at 1x10^5 cells/ml and pre-incubated for 12 h (HL-60) or 24 h (MDA). APC (IC50) was added and cells were incubated for an additional 48 h. Cell numbers were determined using a CASY-1-cell counter, cellular lipids of 1x10^5 cells were extracted, separated by HPTLC and stained with CuSO4-solution (1=control, 2=S-O-acetyl, 3=R-OH, 4=S-OH, 5=R-OH, 6=HePC).
(93% of control). The amount of PE also increased in the HL-60 cells after HePC-incubation (120% of control).

In contrast to HePC and S-configured APC, the less cytotoxic R-configured APC reduced PC, as well as SM by up to 50% in the MDA-MB-468 cells and by 73% in the HL-60 cells (Figure 2, Table III). With the decrease in PC a "new lipid" spot could be simultaneously detected on the HPTLC plate (Figure 2). This "new lipid" had a slightly higher Rf-value than that of PC and its relative amount was similar to the observed reduction of PC.

To identify the "new lipid", attempts were made to separate it from PC and other phospholipids by HPTLC. Unfortunately, complete separation was not possible because of the similar biophysical properties with PC. Thus, both, the PC and the "new lipid" were isolated from other cellular lipids of HL-60 cells by HPTLC, were hydrolysed completely using perchloric acid and the hydrolysed lipids were separated by a second HPTLC-run (Figure 3). Interestingly, octadecandiol could be identified only in the cells previously incubated with the R-configured APC. These results indicate that both R-OH, as well as R-O-acetyl (probably after deacylation to R-OH) were acylated to R-1-O-phosphocholine-2-O-acyl-octadecane (R-O-acyl).

Quantification of the density of the fatty acids and octadecandiol spots showed, in comparison with normal cultivated HL-60 cells, that roughly 80% of the PC-content was replaced by R-O-acyl (Figure 3).

To further prove the role of R-OH and R-O-acetyl as inhibitors of lyso-PC-reacylation, the acylation of radiolabelled $^{14}$C-lyso-PC to $^{14}$C-PC in HL-60 and MDA-MB-468 cells with or without the influence of the APC (IC$_{50}$) were invest-tigated. In the absence of APC (Figure 4), the HL-60 cells showed a almost linear kinetic of acylation for 24 h (Figure 4A). While, for the first few hours MDA-MB-468 cells showed linear kinetics for the $^{14}$C-PC intake, the absolute ratio of acylation was 13-fold higher than in the HL-60 cells. As a consequence, a lack of $^{14}$C-lyso-PC and a decreased velocity of $^{14}$C-PC production could already be detected after 4 h of incubation (Figure 4A). Taking into consideration both the ratio of phospholipid content (HL-60/MDA = 1:3.4) (34) and that of the cell doubling times (HL-60/MDA = 1:0.7) a relative reacylation velocity in the MDA-MB-468 cells of at least 5-fold higher than in the HL-60 cells was calculated. This calculation implicates that either the activity of reacylating enzymes, the amount of reacylating enzymes or the velocity of lyso-PC-uptake differ between the cell lines.

**Table III. Percentage of SM and PC content.**

<table>
<thead>
<tr>
<th>Phospholipids</th>
<th>HL-60 cells</th>
<th>MDA-MB 468 cells</th>
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<tbody>
<tr>
<td></td>
<td>R-O-acetyl</td>
<td>R-OH</td>
</tr>
<tr>
<td>Sphingomyelin (SM)</td>
<td>78%</td>
<td>67%</td>
</tr>
<tr>
<td>Phosphatidylycholine (PC)</td>
<td>79%</td>
<td>27%</td>
</tr>
</tbody>
</table>

HL-60 and MDA-MB-468 cells incubated with R-(natural)-configured APC (IC$_{50}$) and referred to the SM and PC content of the control cells.
The acylation of $^{14}$C-lyso-PC to $^{14}$C-PC after incubation with the APC is shown in Figure 5. Due to the high rate of acylation, the MDA-MB-468 cells were only incubated for 3 h, whereas the HL-60 cells were incubated for 20 h. R-OH and R-O-acetyl reduced $^{14}$C-PC synthesis in HL-60 cells (28% and 65% of control) as well as in MDA-MB-468 cells (50% and 56%). The extent of reductions were very similar to the reductions of total PC found in the previous experiments (Figure 2). These results obtained by using different techniques confirm that R-OH and R-O-acetyl are inhibitors of lyso-PC-acylation.

Discussion

In several studies the antitumoural and antiparasitic efficacy of APC, especially of HePC, could be demonstrated in vitro and in vivo (10-15). Unfortunately, the therapeutic index of HePC is limited due to its severe side-effects (16). In order to improve antineoplastic activity and reduce side-effects, a group of new APC were synthesised (Figure 1). Compared to HePC, these new APC contain additional functional groups (acetic ester and hydroxyl groups) and are expected to increase interaction with lipid-metabolising enzymes, such as acylating enzymes or lysophospholipases.

In this study the antitumoral efficacy of the R-(natural) and S-(unnatural) configured new APC was tested in comparison with HePC. Their influence on phospholipid metabolism was also investigated, focusing on the acylation of lyso-PC to PC.

As initially expected, especially the S-configured APC showed considerably higher cytotoxic activities than HePC. We assume that their higher cytotoxic potential is due to slower metabolism by phospholipases causing higher APC concentrations in cell membranes and increase of apoptosis at the level of CD95 (20, 29, 30).

Furthermore, our data indicate that APC bearing an acetic ester group (R/S-O-acetyl) displayed higher cytotoxic activities than APC bearing a hydroxyl group (R/S-OH). This could be due to a lower polarity (lower CMC-values) of R/S-O-acetyl leading to a preferential accumulation of both ester compounds in cell membranes.

Interestingly, the cytotoxic activity (LC$_{50}$-values) of the tested compounds was generally higher for MDA-MB-468 cells than for HL-60 cells. However, concerning the inhibition of metabolic activity (IC$_{50}$-values) the results differ considerably (Table I). Although APC bearing a hydroxyl group (R/S-OH) inhibited the activity of the respiratory chain in the MDA-MB-468 cells (IC$_{50}$) to a higher degree than in the HL-60 cells, this phenomenon was not associated with an increase of cell death. However, the explanation for the observed effect is unclear. It could be speculated that R/S-OH accumulated in the

Figure 3. Identification of R-O-acyl in HL-60 cells after incubation with R-OH (IC$_{50}$): HL-60 cells were seeded at 1x10$^5$ cells/ml and pre-incubated for 12 h. R-OH (IC$_{50}$) was added and cells were incubated for an additional 48 h. The cellular lipids were extracted and separated by HPTLC. The PC/new lipid-spot was scratched from the plate and the lipids were totally hydrolysed using perchloric acid (A). The resulting solution was extracted and the products were again separated by HPTLC (B); (1=PC; 2=R-O-acyl; 3=fatty acid; 4=octadecadiol; 5=total hydrolysis of the PC-spot of control cells; 6=total hydrolysis of the PC/new lipid-spot of cells incubated with R-OH).
mitochondrial membranes of the MDA-MB-468 cells. However, specific interactions of R/S-OH with mitochondria membrane lipid metabolising enzymes are not probable, because both compounds cause similar inhibition of cellular respiration.

In order to investigate the influence of the new APC on phospholipids metabolism, we analysed the phospholipid composition of HL-60 and MDA-MB-468 cells after incubation for 48 h. While the MDA-MB-468 cells showed no significant difference in phospholipid composition after

Figure 4. Reacylation of 14C-LPC to 14C-PC. Reacylation of 14C-lyso-PC to 14C-PC in HL-60 cells (■) and MDA-MB-468 cells (◆) over 24 h in the absence of APC. HL-60 and MDA-MB-468 cells were seeded at 1x10⁵ cells/ml and pre-incubated for 12 h (HL-60) or 24 h (MDA). 14C-lyso-PC was added and cells were incubated again. HL-60 for 3, 6, 12 h; MDA for 3, 6, 12 h. Afterwards, cells were counted using a CASY-1-cell counter, cellular lipids were extracted and separated by HPTLC. 14C-PC (A) and 14C-lyso-PC (B) were quantified using a phosphoimager. All experiments were done in triplicate.

Figure 5. Quantification of 14C-PC in HL-60 and MDA-MB-468 cells after incubation with 14C-lyso-PC and APC (IC₅₀): HL-60 and MDA-MB-468 cells were seeded at 1x10⁵ cells/ml and pre-incubated for 12 h (HL-60) or 24 h (MDA). APC (IC₅₀) and 14C-lyso-PC were added and cells were incubated for an additional 20 h (HL-60) or 3 h (MDA). Afterwards, cells were counted using a CASY-1-cell counter, cellular lipids were extracted and separated by HPTLC. 14C-PC was quantified using a phosphoimager. All experiments were done in triplicate (1=control; 1=HePC; 3=S-O-acetyl; 4=R-O-acetyl; 5=S-OH; 6=R-OH).
incubation with HePC and the S-configured ALP, an increase of PE up to 150% in the HL-60 cells could be determined. The reason for the increase of PE is not clear. Because PE has membrane stabilising biophysical properties we assume that the cells try to compensate for the destabilising effect of APC. As APC are micelle-forming lipids, their packing parameters are expected to be similar to lyso-PC (<0.7). In contrast, PE are lipids which form inverted micelles since their packing parameters are >1.3. It is known from biophysical studies that mixtures of lyso-PC and PE form stable bilayer membranes, because their average packing parameters are approximately 1.0 (31).

In contrast to the S-configured APC, an enormous reduction of PC and SM in cell membranes could be detected after incubation with R-configured APC. Surprisingly, the reduction of PC and SM was not accompanied by an increase in akute cytotoxicity. This was amazing because PC and SM are important membrane stabilising phospholipids and represent more than half of the total lipid content of both cell lines (32-34). Interestingly, together with the decrease of PC and SM a "new lipid" could be detected in the membranes of both cell lines, identified as R-O-acyl. Further investigation demonstrated the conversion of R-OH or R-O-acetyl (after deacetylation) to R-O-acyl. Moreover, our data implicate not only that R-O-acyl synthesis correlated inversely with the loss of PC in cell membrane but also that R-OH and R-O-acyl (after deacetylation) are substrates for acylating enzymes in cell membrane and compete with lyso-PC for acylation. Similar to PC, R-O-acyl bears two long non-polar hydrocarbon chains and is therefore expected to have comparable biophysical properties. Thus, the enrichment of R-O-acyl in cell membrane after incubation with R-configured APC is expected to compensate the loss of even high amounts of PC structurally. Further investigations with sPLA2 revealed that R-O-acyl is deacylated much more slowly than PC (data not shown). Therefore, we assume that the exchange of PC with R-O-acyl up to 70% in the tumour cell membrane inhibits the intracellular lipid signalling considerably. Thus, in contrast to S-configured APC, which induce apoptosis effectively, the antitumoral efficacy of R-configured APC might partly be explained by R-O-acyl membrane enrichment.

In summary, we demonstrated that the new APC, which are structurally closer to lyso-PC than HePC, show antineoplastic activities similar to or higher than HePC in vitro. However, only R-(natural)-configured APC revealed significant changes in phospholipid composition characterised by an increase in R-O-acyl and loss of PC up to 70% in the HL-60 and MDA-MB 468 cells. Similar to PC, R-O-acyl bears two long non-polar hydrocarbon chains and is, therefore, expected to have comparable structural properties and less side-effects in vivo. Despite their immediate acylation to R-O-acyl and lack of induction of apoptosis, the R-configured APC revealed remarkable antitumoral effects in vitro, which might be caused partly by the inhibition of intracellular lipid signalling. Further investigations are necessary, not only to investigate the influence of R-O-acyl on intracellular cell signalling, but also to elucidate the antitumoral efficacy and toxicity of the new APC in vivo.

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