Cancer Chemother Pharmacol (1992) 30: 105–112

Cancer Chemotherapy and Pharmacology

Alkylphosphocholines: influence of structural variation on biodistribution at antineoplastically active concentrations*

Jochem Kötting[1], Martin R. Berger[2], Clemens Unger[3], and Hansjörg Eibl[1]

[1] Max-Planck-Institut für biophysikalische Chemie, Am Fassberg, D-3400 Göttingen, FRG
[2] Deutsches Krebsforschungszentrum, Institut für Toxikologie u. Chemotherapie, Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG
[3] Medizinische Universitätsklinik, Abt. Hämatologie/Onkologie, Robert-Koch-Strasse 40, D-3400 Göttingen, FRG

Received 14 September 1991/Accepted 23 January 1992

Summary. Hexadecylphosphocholine (HPC) and octadecylphosphocholine (OPC) show very potent antitumor activity against autochthonous methylnitrosourea-induced mammary carcinomas in rats. The longer-chain and unsaturated homologue erucylphosphocholine (EPC) forms lamellar structures rather than micelles, but nonetheless exhibits antineoplastic activity. Methylnitrosourea was used in the present study to induce autochthonous mammary carcinomas in virgin Sprague-Dawley rats. At 6 and 11 days following oral therapy, the biodistribution of HPC, OPC and EPC was analyzed in the serum, tumor, liver, kidney, lung, small intestine, brain and spleen of rats by high-performance thin-layer chromatography. In contrast to the almost identical tumor response noted, the distribution of the three homologues differed markedly. The serum levels of 50 nmol/ml obtained for OPC and EPC were much lower than the value of 120 nmol/ml measured for HPC. Nevertheless, the quite different serum levels resulted in similar tumor concentrations of about 200 nmol/g for all three of the compounds. Whereas HPC preferably accumulated in the kidney (1 μmol/g), OPC was found at increased concentrations (400 nmol/g) in the spleen, kidney and lung. In spite of the high daily dose of 120 μmol/kg EPC as compared with 51 μmol/kg HPC or OPC, EPC concentrations (100–200 nmol/g) were low in most tissues. High EPC concentrations were found in the small intestine (628 nmol/g). Values of 170 nmol/g were found for HPC and OPC in the brain, whereas the EPC concentration was 120 nmol/g. Obviously, structural modifications in the alkyl chain strongly influence the distribution pattern of alkylphosphocholines in animals. Since EPC yielded the highest tissue-to-serum concentration ratio in tumor tissue (5.1) and the lowest levels in other organs, we conclude that EPC is the most promising candidate for drug development in cancer therapy.

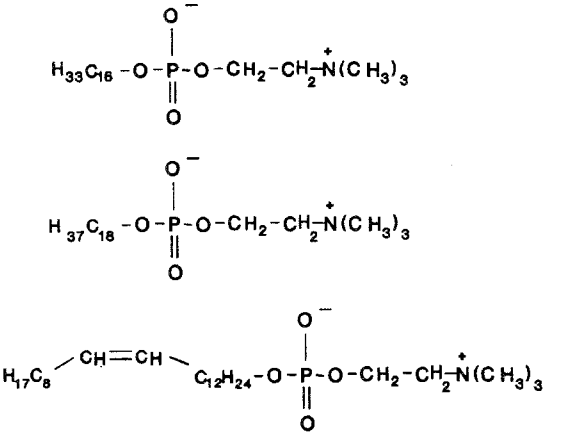


Fig. 1 a–c. Structures of the APCs tested in this study: **a** HPC, **b** OPC, **c** EPC

Introduction

Alkylphosphocholines (APCs) are a new group of biologically active compounds with remarkable antineoplastic activity [9, 23] that were originally derived from alkylglycero phosphocholines and analogues. Due to their lack of glycerol, they show a completely different pattern of biological effects than does platelet-activating factor (PAF) [14, 29]. The first APC intensively investigated was hexadecylphosphocholine (HPC), (Fig. 1 a), which produces a selective and dose-dependent reduction of tumor-cell growth in vitro and in vivo [11, 24, 26]. A strong antitumor effect against methylnitrosourea (MNU)- and 7,12-dimethylbenzanthracene-induced autochthonous mammary carcinoma in rats has also been demonstrated [3, 12, 17], leading to intensive study of the antitumor effects of APCs in these models [2, 30]. These results paved the way for the introduction of HPC into clinical phase I/II trials [22, 25].

The mode of action of HPC remains unknown. Recently, its ability to induce cellular differentiation in leukemic cell lines was shown [13]. The toxicity profile of

* This work was supported by the Deutsche Forschungsgemeinschaft and by a grant from the Bundesministerium für Forschung und Technologie

Offprint requests to: J. Kötting, MPI für biophysikal. Chemie, Am Fassberg, D-3400 Göttingen, FRG

Table 1. Dosing scheme for the different APCs tested to induce the regression of MNU-induced mammary carcinomas in female rats

Group	Treatment[a]	Number of animals	Dose		Administration route
			mg/kg	μmol/kg	
1	Control	6	–	–	–
2	HPC	6	21	51	p.o.
3	OPC	6	22	51	p.o.
4	EPC	6	59	120	p.o.

[a] Daily treatment 5 times a week. Subgroups of 3 rats were formed after 6 and 11 applications, respectively

HPC is completely different from that of classic carcinostatic agents, especially with regard to myelosuppression, the most noticeable and consistent toxicity produced by chemotherapeutic agents [4], in that HPC has a stimulatory effect on hematopoiesis [12]. The first information on the pharmacokinetics of HPC and its organ distribution was obtained using radiolabeled HPC [5]. Radioactivity was found mainly in kidney, lung and liver tissues, but the chemical integrity of HPC was not confirmed in these experiments. Subsequently, serum concentrations of HPC in rats were determined using high-performance liquid chromatography [27]. Prior to the introduction of HPC into clinical phase I/II trials, it was important to improve our knowledge about the distribution of this drug in the different tissues and to understand possible correlations between its efficacy and its side effects. We successfully developed a method for quantitative determination of HPC and its analogues using high-performance thin-layer chromatography (HPTLC) as based on a previous procedure [21]. The lower limit of detection for APCs in tissues is about 1 nmol/g [16].

This is the first report to provide a comparison of serum levels of different APCs with their concentrations in organs and tumor tissue. Rats bearing MNU-induced mammary carcinomas were treated with HPC, octadecylphosphocholine (OPC, Fig. 1b) and erucylphosphocholine (EPC, Fig. 1c). Serum and tissue concentrations were determined using HPTLC in animals that showed marked tumor regression as early as after 6 and 11 days of therapy.

Materials and methods

Alkylphosphocholines. Synthetic routes for the preparation of these compounds (Fig. 1) have been described elsewhere [10, 28].

Animals and tumor induction. Female virgin Sprague-Dawley rats (Institut für Versuchstierkunde, Hannover, FRG) supplied at the age of 40 ± 1 days were kept under conventional controlled conditions (three animals per size III Makrolon cage during the tumor-induction period and, subsequently, one animal per size II Makrolon cage during therapy; $22° \pm 2°$ C; relative humidity, $55\% \pm 10\%$). Altromin pellets and tap water were given ad libitum. Mammary carcinomas were induced according to Berger et al. [1] by three i.v. injections of 50 mg/kg MNU into the tail vein of rats aged 50, 71 and 92 days. At 6 weeks following the first injection of MNU, rats were weighed and palpated twice weekly throughout the experimental period to evaluate tumor development. Individual tumor volumes were estimated as the product of two vertical axes $(a \times b^2)/2$ as measured using Vernier calipers (a>b). Rats showing a total tumor volume of greater than 0.8 cm^3 were randomly allocated to experimental groups and immediately underwent the first application of the different APCs.

Therapy and dosage. APCs were dissolved in distilled water and given orally by gastric intubation. The experimental design is shown in Table 1. On the basis of preliminary studies, doses were chosen that resulted in similar antitumor responses to the three APCs. The respective doses were given on 5 consecutive days a week, weekends being omitted. Therapy was terminated in 3 animals from each group at 24 h after the 6th or 11th application, respectively. Antitumor activity was assessed by recording the number of discrete tumors and the mean tumor volume per rat until the end of the study. For determinations of tumor, serum and organ levels of APCs, animals under ether anesthesia were killed by exsanguination. Serum was obtained from blood by centrifugation. Tumor, liver, spleen, kidney, lung, brain, and small-intestine tissues were excised and shock-frozen in liquid nitrogen; all samples were stored at –70° C.

Analysis of APCs in sera and tissues. The quantitative determination of different APCs in rat serum and tissues was performed using the HPTLC method described elsewhere [16]. In short, tissue samples (0.1–0.5 g) were homogenized with $H_2O/CHCl_3$ (1 : 1, v/v) in a motor-driven Potter-Elvehjem homogenizer (3000 rpm); subsequently, total lipid extraction with 2 ml $CHCl_3/CH_3OH$ (2 : 1, v/v) was performed and repeated twice. The combined organic extracts were dried under a gently stream of nitrogen. The lipids were dissolved in $CHCl_3/CH_3OH/H_2O$ (30 : 60 : 8, by vol.) and spotted onto HPTLC plates (silica gel 60, Merck, Darmstadt, FRG). They were developed in $CHCl_3/CH_3OH$/triethylamine/H_2O for analysis of HPC/OPC (30 : 35 : 34 : 8, by vol.) and for EPC (30 : 50 : 34 : 8, by vol.). The plates were dried at 180° C, stained by dipping in a 10% solution of $CuSO_4$ in 8% H_3PO_4, and quantitated in a CD60 densitometer (Desaga, Heidelberg, FRG) [21]. Pure APCs were used as quantitative standards on each plate.

Results

Inhibition of tumor growth

The total observation period comprised 11 days. Within this time, the tumor volume and the number of tumors in untreated rats increased from 0.8 cm^3 and two to four tumors per rat to 3–9 cm^3 and five to nine lesions per rat, respectively (Fig. 2a). On the basis of preliminary experiments, doses of HPC, OPC and EPC were chosen that would cause at least partial arrest or partial regression of tumor growth in MNU-induced mammary carcinomas. At the doses shown in Table 1, this situation was achieved after 11 days of treatment; at this point, enough tumor material remained available for analysis of APC concentrations in tumor tissue. HPC (Fig. 2b), OPC (Fig. 3a) and EPC (Fig. 3b) were similarly effective at these doses, showing increasing efficacy in a comparison of single rats after 6 and 11 applications. HPC, OPC and EPC also completely inhibited the manifestation of new tumors.

APC analysis in tissues

Rats with MNU-induced tumors were treated with the three different APCs according to the schedule described in Materials and methods. After 6 days of treatment, each animal had received a total dose of 96 μmol HPC or OPC but 231 μmol EPC (also see Table 1). After 11 days,

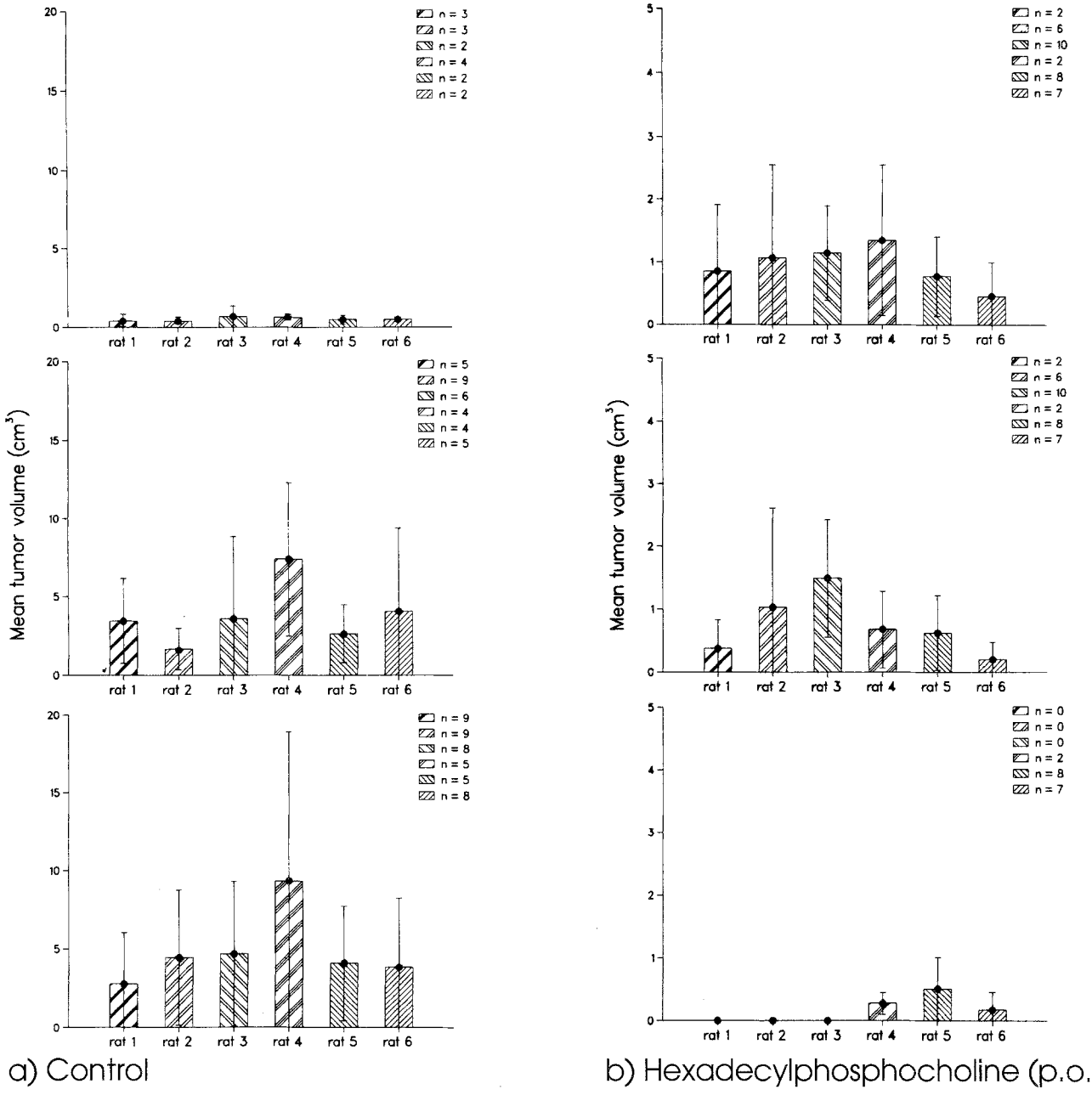


Fig. 2 a, b. Tumor regression caused by HPC in the MNU-induced mammary carcinoma model in rats **b)** as compared with **a** an untreated control group **a)**. The numbers of tumors and their volumes in each individual animal were estimated before therapy with HPC (*top*), after 6 days of treatment (*middle*) and after 11 days (*bottom*) as described in Materials and methods. Note the different scales for the ordinates of **a)** and **b)**

176 μmol HPC or OPC but 424 μmol EPC had been given in total. The biodistribution of the compounds in the serum, liver, kidney, lung, small intestine, brain and spleen of rats is shown in Figs. 4–7. The remaining organs did not contain significant amounts of APCs and are not included. The overall recoveries for the respective APCs ranged between 20% (6 days) and 8% (11 days) as calculated from the sum of the organ contents.

Under these conditions, the serum concentrations of HPC reached a constant level of 120 nmol/ml over the whole observation period (Fig. 4a). This is the steady-state level of HPC as estimated in previous 4-week studies [15, 27]. Pronounced tumor regression (Fig. 2b) was observed along with a HPC concentration in the tumor (Fig. 4b) of about 180 nmol/g. Additional application of HPC for 5 further days did not lead to alterations in the HPC content of the tumor, although tumor regression continued (Fig. 2b). In contrast, HPC uptake in the liver continuously increased from 272 nmol/g after 6 days to 410 nmol/g (Fig. 5a) after 11 days. By far, the highest concentration of HPC was found in the kidney (Fig. 5b); after 6 days, a level of about 1300 nmol/g was detected. This dropped to 850 nmol/g after 11 days. Lung tissue was another main target of HPC, with concentrations of greater than 500 nmol/g being measured after 11 days (Fig. 6a). In the small intestine (Fig. 6b) and in the brain (Fig. 7a),

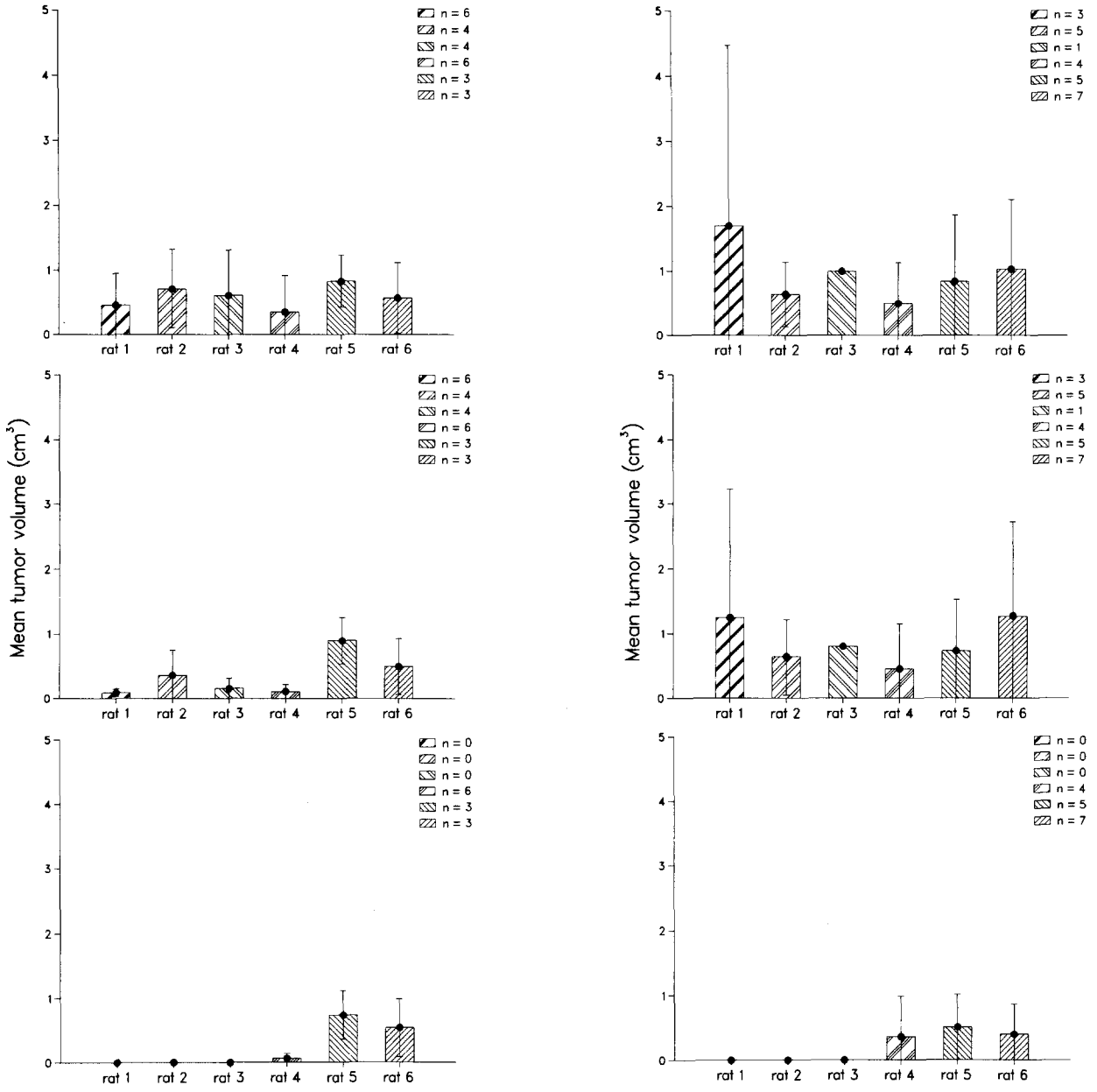
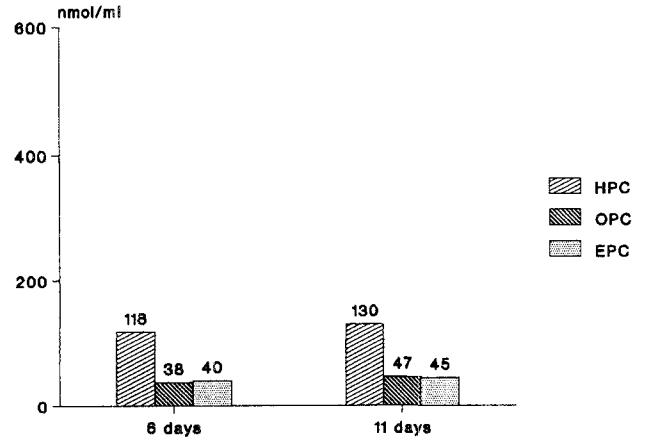


Fig. 3 a, b. Tumor regression caused by OPC **a)** and EPC **b)** treatment of MNU-induced mammary carcinomas in rats. The numbers of tumers and their mean volumes in each individual animal were estimated before therapy with the compounds (*top*), after 6 days of treatment (*middle*) and after 11 days (*bottom*) as described in Materials and methods

HPC concentrations increased by a factor of 3 (from day 6 to day 11).

The organ distribution of OPC was somewhat different from that of HPC. The OPC concentration in serum was only one-third that of HPC (Fig. 4a). The tumor concentrations of these two agents did barely change from week 1 to week 2 as shown in Fig. 4b. Tumor regression was also observed using OPC (Fig. 3a). The level of OPC in liver tissue (Fig. 5a) was one-third lower than that of HPC after 11 days. In general, OPC concentrations in all tissues did not vary greatly; however, in the spleen (Fig. 7b), the concentration of OPC (424 nmol/g) was higher than that of HPC or EPC and reached the highest peak of all three APCs.

The EPC serum concentration of 45 nmol/ml was comparably as low as that observed for OPC (Fig. 4a). This value was sufficient to supply the MNU-induced tumor with a concentration of 230 nmol/g (Fig. 4b) over the period studied. To reach this concentration of EPC in tissues, higher doses had been given to the animals (Table 1). However, with the exception of the tumor, the lung and the small intestine, the levels of EPC in all tissues were significantly lower than those of HPC or OPC after 11 days of treatment.



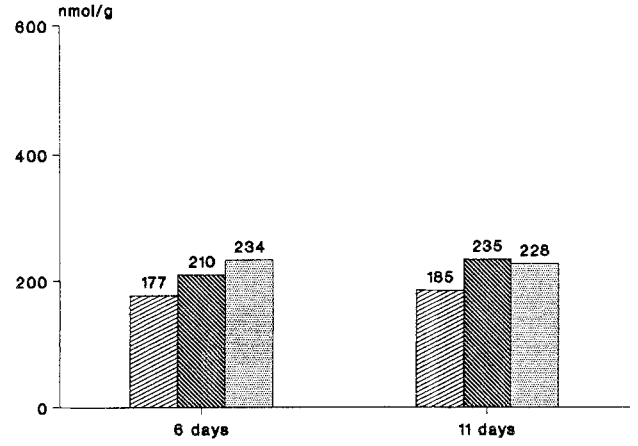
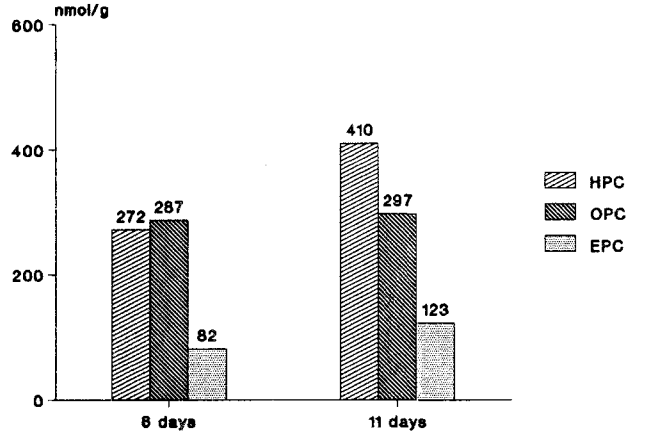


Fig. 4 a, b. Concentrations of HPC, OPC and EPC in **a** serum and **b** tumors of rats bearing MNU-induced mammary carcinomas (see Figs. 2 b, 3 a, b). APC concentrations were analyzed by HPTLC as described in Materials and methods. The concentrations (given in nmol APC/g tissue) represent mean values for 3 estimations obtained in 3 animals from each group and for each time point (SD, within ±20%)



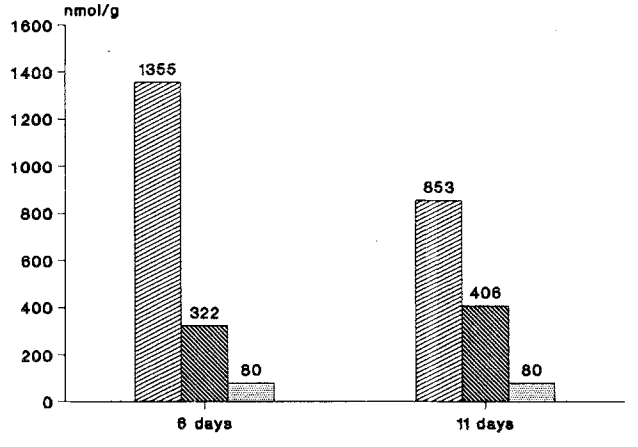
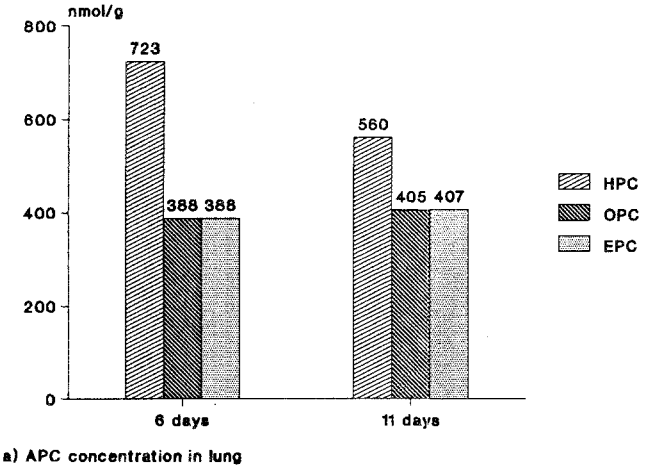


Fig. 5 a, b. Concentrations of HPC, OPC and EPC in **a** livers and **b** kidneys of rats bearing MNU-induced mammary carcinomas (see Figs. 2 b, 3 a, b). APC concentrations were analyzed by HPTLC as described in Materials and methods. The concentrations (given in nmol APC/g tissue) represent mean values for 3 estimations obtained in 3 animals from each group and for each time point (SD, within ±20%)



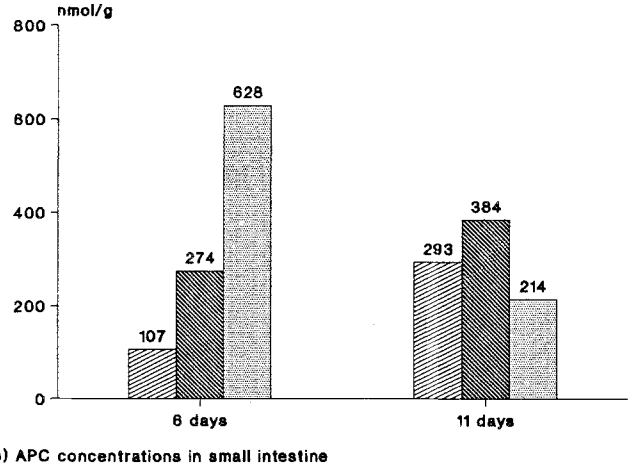


Fig. 6 a, b. Concentrations of HPC, OPC and EPC in **a** lungs and **b** small intestines of rats bearing MNU-induced mammary carcinomas (see Figs. 2 b, 3 a, b). APC concentrations were analyzed by HPTLC as described in Materials and methods. The concentrations (given in nmol APC/g tissue) represent mean values for 3 estimations obtained in 3 animals from each group and for each time point (SD, within ±20%)

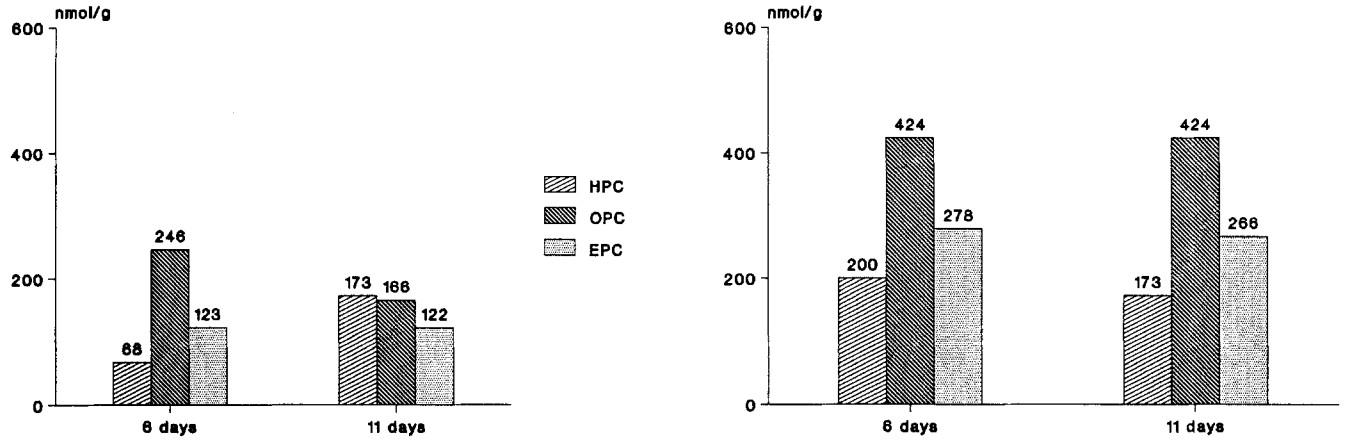


Fig. 7 a, b. Concentrations of HPC, OPC and EPC in **a** brains and **b** spleens of rats bearing MNU-induced mammary carcinomas (see Figs. 2b, 3a, b). APC concentrations were analyzed by HPTLC as described in Materials and methods. The concentrations (given in nmol APC/g tissue) represent mean values for 3 estimations obtained in 3 animals from each group and for each time point (SD, within ±20%)

Table 2. Enrichment factors in tissues of the APCs HPC, OPC and EPC in the organs of rats bearing MNU-induced tumors as compared with serum concentrations after 11 days of oral treatment with the respective compound

APC	Tissue						
	Tumor	Liver	Kidney	Lung	Brain	Small intestine	Spleen
HPC	1.4	3.2	6.6	4.3	1.3	2.3	1.3
OPC	5.0	6.3	8.6	8.6	3.5	8.2	9.0
EPC	5.1	2.7	1.8	9.0	2.7	4.8	5.9

The average tissue-to-serum ratios following treatment with APCs are shown in Table 2. For instance, a value of 1.6 for EPC in the kidney indicates that the concentration in the organ was 1.6 times higher than that in serum. The highest ratios between tumor and serum were observed for EPC and OPC at values of 4.6 and 4.4, respectively, whereas the value of 1.5 obtained for HPC after 11 days was low. Generally, the transfer of EPC from serum to tissue and especially to tumor was more highly favoured than that of HPC. OPC showed the highest enrichment factor in all tissues, but that of EPC was similar, if not slightly higher, in the tumor. However, apart from its low ratio, the absolute quantity of HPC in the tumor did not differ from that of OPC or EPC.

Discussion

APCs represent a new group of antitumor drugs that were originally introduced by Eibl and Unger [7, 8, 22]. Berger et al. [3] and Muschiol et al. [17] demonstrated that these compounds show strong and selective antineoplastic activity in vivo in the treatment of MNU-induced mammary carcinomas. HPC and OPC caused complete tumor regression in about 30% of animals after oral application. As expected from previous studies using dodecylphosphocholine, tetradecylphosphocholine and HPC [3], the antineoplastic activity of APCs correlated with the chain length of the alkyl moiety. No effect was observed for dodecylphosphocholine and marginal activity for tetradecylphosphocholine, but HPC and OPC strongly reduced the tumor burden of the animals [3]. It should be emphasized that in these early studies, HPC caused less toxicity in the animals than did OPC; therefore, the former is the favoured candidate for clinical investigations. The therapeutic efficacy of EPC (Fig. 3b) was comparable with that of HPC and OPC. However, a higher oral dose of EPC was necessary to obtain tumor regression similar to that achieved using HPC or OPC, thus prompting interest in the fate of the drug with respect to absorption and metabolism. Phospholipids are absorbed by the gastrointestinal tract as lysolipids [6, 19]. HPC and OPC are similar to lysolecithins in their physical properties, forming micelles at critical micellar concentrations of 8×10^{-6} (HPC) and 3.4×10^{-6}M (OPC) [18]. EPC differs from HPC and OPC in that it forms lamellar structures rather than micelles.

A 4-week study of the subacute toxicity of APCs in rats following their intragastric application revealed that the absorption of HPC and OPC was almost complete [15]. However, only 90% uptake was measured for EPC [20]. The true bioavailability of HPC and OPC cannot be estimated by comparing p.o. and i.v. concentrations, since i.v. administration of HPC and OPC is possible only using liposomal carriers. No saturation of absorption was observed over the 4-week study period. The different time courses observed for HPC, OPC and EPC accumulation in the small intestine during our 6- and 11-day study suggest that the compounds show different absorption behavior. For HPC, a 3-fold enrichment in the small intestine was observed between day 6 (107 nmol/g) and day 11 (293 nmol/g). OPC was incorporated into the small intestine much faster than was HPC, with a large increase in concentration being observed after day 6 (314 nmol/g) and

a further increase up to day 11 being marginal (384 nmol/g). The behavior of EPC was completely different. High initial concentrations in the small intestine after day 6 (826 nmol/g) indicated slow transport into the other tissues. Surprisingly, after 11 days of treatment, the EPC concentration in the small intestine was markedly reduced (214 nmol/g). At present, this finding is not understood and needs further examination. As shown in Figs. 4–7, almost no increase in EPC concentration occurred from day 6 to day 11. This would be the result of rapid degradation of EPC in the gastrointestinal tract, which would also explain the high EPC dose required to induce tumor regression. However, the metabolism of HPC (half-life, 96 h) is usually remarkably slow [27].

The aim of this study was to analyze the organ distribution of APCs exhibiting different alkyl chains under experimental conditions that result in very similar antineoplastic effects in rats bearing MNU-induced mammary carcinoma. This goal was achieved perfectly as shown in Figs. 4–7, since the observed effects on the size of the tumors were quite similar for all three of the compounds and the HPC, OPC and EPC concentrations achieved in the tumor were just about the same (200 nmol/g). Since the same amount of the different APCs in tumor tissue led to an almost identical response, the micellar or lamellar superstructure of the compounds does not seem to be important for the in vivo antitumor effect of these agents. However, the length of the alkyl chain seemed to be important for the quite differing tissue distribution of the three APCs.

In summary, the distribution of the three APCs in rats shows that HPC is preferentially enriched in the kidney, lung and liver, whereas OPC accumulates in the lung, kidney, and small intestine. Finally, EPC differs from HPC and OPC in that its only potential target tissue was the lung. Surprisingly, this compound concentrates in the MNU-induced tumor. Therefore, two important questions have to be answered. First, do models of other primary tumors also concentrate EPC and is such a concentration reflected in the tumor response. Second, do different tissue levels of structurally modified APCs also lead to an acceptable toxicity profile?

References

1. Berger MR, Habs M, Schmähl D (1983) Noncarcinogenic chemotherapy with a combination of vincristine, methotrexate and S-fluorouracil (VMF) in rats. Int J Cancer 32: 231–236
2. Berger MR, Muschiol C, Schmähl D, Eibl H (1987) New cytostatics with experimentally different toxic profiles. Cancer Treat Rev 14: 307–317
3. Berger MR, Muschiol C, Schmähl D, Unger C, Eibl H (1987) Chemotherapeutische Studien zur Struktur-Wirkungs-Beziehung zytotoxischer Alkyllysophospholipide an chemisch induzierten Mammakarzinomen der Ratte. In: Nagel GA, Sauer R, Schreiber HW (eds) Die Zellmembran als Angriffspunkt der Tumortherapie, vol 34. W. Zuckschwerdt, München, pp 27–36
4. Berger MR, Osswald H, Schmähl D, Zeller WJ (1990) Krebschemotherapie unter Berücksichtigung präklinischer Grundlagen. In: Kümmerle W, Hitzenberger H, Spitzer K (eds) Klinische Pharmakologie, 4th, edn., vol 27. Ecomed, pp 1–50
5. Breiser A, Kim DJ, Fleer EAM, Damenz W, Drube A, Berger MR, Nagel GA, Eibl H, Unger C (1987) Distribution and metabolism of hexadecylphosphocholine in mice. Lipids 22: 925–926
6. Brindley DN (1985) Metabolism of triacylglycerols – biochemistry of lipids and membranes, vol 7. Benjamin/Cummings, Menlo Park, California, pp 221–229
7. Eibl H, Unger C (1987) Phospholipide als Antitumormittel: Möglichkeiten einer selektiven Therapie. In: Unger C, Eibl H, Nagel GA (eds) Die Zellmembran als Angriffspunkt der Tumortherapie, vol 34. W. Zuckschwerdt, München, pp 1–18
8. Eibl H, Unger C (1989) Biologische Bedeutung der Phospholipide: Entwicklung von Hexadecylphosphocholin – Eine Substanz mit selektiv-antitumoralen Eigenschaften. In: Schmähl D, Peukert M (eds) Fortschritte der Medizinischen Forschung: Onkologie. Regensburger Universitätskolloquium 1989. Schattauer, Stuttgart, pp 41–51
9. Eibl H, Unger C (1990) Hexadecylphosphocholine: a new and selective antitumor drug. Cancer Treat Rev 17: 233–242
10. Eibl H, Woolley P (1988) A general synthetic method for enantiomerically pure ester and ether lyso-phospholipids. Chem Phys Lipids 47: 47–53
11. Fleer EAM, Kim DJ, Nagel GA, Eibl H, Unger C (1990) Cytotoxic activity of lysophosphatidylcholine analogues on human lymphoma Raji cells. Onkologie 13: 295–300
12. Hilgard P, Stekar J, Voegeli R, Engel J, Schumacher W, Eibl H, Unger C, Berger MR (1988) Characterisation of the antitumor activity of hexadecylphosphocholine (D 18506). Eur J Cancer Clin Oncol 24: 1457–1461
13. Hochhuth C, Berkovic D, Eibl H, Unger C, Doenecke D (1990) Effects of antineoplastic phospholipids on parameters of cell differentiation in U937 cells. J Cancer Res Clin Oncol 116: 459–466
14. Kötting J, Fleer EAM, Unger C, Eibl H (1988) Synthetische Alkyllysophospholipide als Antitumormittel-Strukturverwandte des "Platelet Activating Factors". Fat Sci Technol 90: 345–351
15. Kötting J, Marschner NW, Unger C, Eibl H (1992) Hexadecylphosphocholine and octadecyl-methyl-glycero-3-phosphocholine: a comparison of hemolytic activity, serum binding and tissue distribution. In: Eibl H, Hilgard P, Unger C (eds) Alkylphosphocholines: new drugs in cancer therapy. S. Karger, Basel, pp 131–142
16. Kötting J, Marschner NW, Unger C, Eibl H (1992) Determination of alkylphosphocholines in biological fluids and tissues. In: Eibl H, Hilgard P, Unger C (eds) Alkylphosphocholines: new drugs in cancer therapy. S. Karger, Basel, pp 6–11
17. Muschiol C, Berger MR, Schuler B, Scherf HR, Garzon FT, Zeller WJ, Unger C, Eibl H, Schmähl D (1987) Alkylphosphocholines: toxicity and anticancer properties. Lipids 22: 930–934
18. Neumüller W (1988) Biosynthese von Phospholipiden: Über die Bedeutung acylierender Systeme in Rattenleber. Dissertation, Technische Universität Braunschweig
19. Parthasarathy S, Subbaiah PV, Ganguly I (1974) The mechanism of intestinal absorption of phosphatidylcholine in rats. Biochem J 140: 503–508
20. Reitz RC, Kötting J, Unger C, Eibl H (1992) Comparison of the tissue distribution of hexadecylphosphocholine and erucylphosphocholine. Lipids (in press)
21. Rustenbeck I, Lenzen S (1990) Quantitation of a new antineoplastic agent, hexadecylphosphocholine. Arch Pharmacol 341 [Suppl]: R111
22. Unger C, Eibl H (1989) Onkologische Therapie mit Phospholipiden. In: Schmähl D, Peukert M (eds) Fortschritte der medizinischen Forschung: Onkologie. Regensburger Universitätskolloquium 1989. Schattauer, Stuttgart, pp 55–59
23. Unger C, Eibl H (1991) Hexadecylphosphocholine: preclinical and first clinical results of a new antitumor drug. Lipids 26: 1412–1417
24. Unger C, Eibl H, Breiser A, Heyden HW von, Engel J, Hilgard P, Sindermann H, Peukert M, Nagel GA (1988) Hexadecylphosphocholine (D 18506) in the topical treatment of skin metastases: a phase I trial. Onkologie 11: 295–296
25. Unger C, Eibl H, Nagel GA, Heyden HW von, Breiser A, Engel J, Stekar J, Peukert P, Hilgart P, Berger M (1989) Hexadecylphosphocholine in the topical treatment of skin metastases: a phase I trial. In:

Queißer W, Fiebig HH (eds) New drugs in oncology. (Contributions to oncology series, vol 37) Karger, Basel, pp 219–223
26. Unger C, Damenz W, Fleer EAM, Kim DJ, Breiser A, Hilgard P, Engel J, Nagel GA, Eibl H (1989) Hexadecylphosphocholine, a new ether lipid analogue. Studies on the antineoplastic activity in vitro and in vivo. Acta Oncol 28: 213–217
27. Unger C, Fleer E, Damenz W, Hilgard P, Nagel G, Eibl H (1991) Hexadecylphosphocholine: determination of serum concentrations in rats. J Lipid Mediators 3: 71–78
28. Woolley P, Eibl H (1988) Synthesis of enantiomerically pure phospholipids including phosphatidyl serine and phosphatidyl glycerol. Chem Phys Lipids 47: 55–62
29. Workman P (1991) Platelet activating factor. Cancer Cells 4: 426
30. Yanapirut P, Berger MR, Reinhardt M, Schmähl D (1991) In vitro investigations on the antineoplastic effect of hexadecylphosphocholine. Drug Res 41: 652–655