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Antineoplastic activity and tolerability of a novel heterocyclic alkylphospholipid, D-20133

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Abstract. Octadecyl-[2-(*N*-methylpiperidinio)ethyl]-phosphate (OMPEP, D-20133), a heterocyclic analogue of hexadecylphosphocholine (MIL), has been synthesized in an attempt to increase the therapeutic range of the parent compound. The antineoplastic activity of the novel alkylphospholipid was compared with that of MIL in dimethylbenz(a)anthracene-induced mammary carcinoma of the rat. Using tumors of different sizes and repeated daily doses as well as high single doses, we achieved marked remissions with either compound. However, the therapeutic range of OMPEP was broader than that of the parent drug. Furthermore, the emetic potential of OMPEP tested on ferrets was distinctly less pronounced than that of MIL. In vitro the new alkylphospholipid proved to be more active than MIL in all cell lines tested, and its differentiation-inducing capacity turned out to be superior to that of MIL. No hematological toxicity was observed at various OMPEP doses during a 3-week treatment period.

Introduction

Like the etherphospholipids (e.g., 1-0-octadecyl-2-0-methyl-rac-glycero-3-phosphocholine, ET-18-OCH3), their congeners the alkylphosphocholines have recently shown interesting antineoplastic properties [2, 12]. Among them, hexadecylphosphocholine (miltefosine, MIL; Fig. 1), one of the most active compounds in this series, proved to be very active in methylnitrosourea- and dimethylbenz(a)anthracene (DMBA)-induced autochtonous carcinomas of the rat [2, 5]. Moreover, in the latter tumor, MIL was more effective than cyclophosphamide [5]. The lack of hematological toxicity was another outstanding property of this compound; contrary to conventional cytostatics, during treatment with MIL, neutrophil granulocytosis was observed in rats [5]. After repeated administration of oral MIL doses in rats, the gastrointestinal tract was shown to be the specific target organ for toxicity [12]. Besides, intense salivation was observed in treated animals, pointing to the possibility that this parasympathomimetic effect was due to the stimulation of cholinergic receptors by some low-molecular-weight derivative(s) of choline formed during MIL metabolism. Such degradation products could also be at least partly responsible for the gastrointestinal toxicity observed in rats. Therefore, in preparing novel MIL congeners, we concentrated on the replacement of choline by other appropriate structures; several heterocyclic nitrogen compounds were used for this purpose. The new alkylphospholipids should be less toxic than the parent compound yet should possess similar antineoplastic activity. Among the resulting compounds, octadecyl-[2-(*N*-methyl-piperidinio)-ethyl]phosphate (OMPEP, D-20133; Fig. 1) showed outstanding properties in pilot experiments on rats with DMBA-induced tumors and was therefore selected for in-depth investigation.

In the present study, a comparison was made between MIL and its new analogue in regard to toxicity and antineoplastic activity using DMBA-induced tumors of different sizes as well as several in vitro systems for testing. Preliminary experiments showed that the MIL-induced damage to the intestine was associated with diminished food intake and a loss in net body weight; the latter parameter served as the basis for comparing the tolerability of the two alkylphospholipids with reference to the doses exerting equal antineoplastic activity in rats bearing DMBA-induced tumors. Since vomiting has been observed in clinical phase I trials of oral MIL [3], we also tested the emetic potential of OMPEP in comparison with that of the parent compound. Finally, MIL has been shown to be a differentiation inducer in vitro [10] and in vivo [6]. These findings prompted us to compare the differentiation-inducing capacity of MIL and OMPEP using leukemic cell lines in vitro.

The first author dedicates this work to the memory of Tone Slivnik

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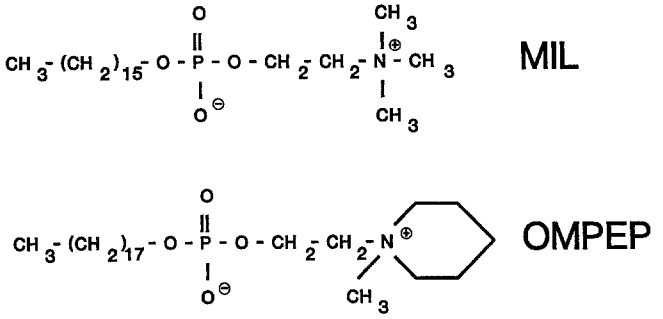


Fig. 1. Chemical structures of hexadecylphosphocholine (*MIL*; molecular weight, 407.6 Da) and octadecyl-[2-(*N*-methyl-piperidinio)-ethyl]phosphate (OMPEP; molecular weight, 475.7 Da)

Materials and methods

Synthesis of OMPEP. The novel alkylphospholipid was synthesized as described previously [14].

Animals and tumor induction. Female Sprague-Dawley rats (Moellegaards Breeding Center, Ejby, Denmark) were used throughout the experiments; adult animals (weighing 220–270 g) were used for toxicology and hematology studies and 50-day-old rats, for experiments with DMBA-induced tumors. Ferrets for emesis experiments were obtained from Grayston Guinea-Pigs (Ringwood, England). For the induction of tumors, a single dose of 20 mg DMBA (Serva, Heidelberg) dissolved in 1 ml olive oil was given by gavage to 50-day-old rats. Approximately 1 month later the first tumors appeared. The tumor weight was estimated by palpation and by comparison of its volume with that of the prefabricated plasticine models as described previously [5].

Treatments and evaluation of toxicity and therapeutic effectiveness. OMPEP and MIL (manufactured by ASTA Medica AG) were dissolved in 0.9% saline and given to animals through a stomach tube; both substances were of a chemical purity of greater than 98%. The oral dose acutely lethal to 50% of the population (LD_{50}) was estimated in several groups of six animals; the evaluation was done by means of probit analysis. For a comparison of the tolerability of the two alkylphospholipids, three dose groups consisting of six animals each were used; the rats were treated by gavage once daily for 11 days. For experiments on hematological toxicity, three groups of five animals were injected with daily oral doses of OMPEP (ranging from 21.5 to 46.6 mg/kg) for 3 weeks. Red cell counts, platelet counts, leukocyte counts, and blood smears were performed using standard techniques. Blood was taken from the sublingual vein of the rat. For assessment of the emetic potential of either compound in ferrets [4], three groups of six animals were treated orally with logarithmically spaced single doses. These studies were performed at the Huntingdon Research Centre Ltd. (England). The animals were observed for 4 h posting, and both the number of animals vomiting and the latency period (time to the onset of vomiting) were recorded.

Rats with large (weight, 4–6 g) or small (0.8–2 g) tumors were randomly allocated to control and test groups (consisting of 6–7 animals each), thus ensuring an approximately equal distribution of tumors with different latencies, total tumor weights and numbers of tumor nodes among the experimental animals. The therapy started immediately thereafter. The doses were logarithmically spaced. After dosing, the animals were observed for an additional 7 or 14 days, depending on the experiment, and the tumor weights were registered. Controls were given 0.9% saline. For assessment of the net body weight (NBW), the tumor weight was subtracted from the total body weight of the rat. To quantify the effect of the treatment on tumor growth, we used the usual formula $\frac{T}{C} \times 100\%$ (T and C referring to the median tumor weight in the treated and control groups respectively). However, for a clear description of the experimental results it was necessary to introduce an additional index, discriminating between the inhibition of growth and the regression of the tumors. This was done by calculating $\frac{m_t}{m_o} \times 100\%$, i.e. the ratio of the median tumor weight observed at a specified time (m_t) and that registered at the beginning of treatment (m_o). Thus, values lower than 100% represent tumor regression and those of 100% and more indicate static and progressive tumors, respectively.

Table 1. Toxic deaths observed in healthy adult female Sprague-Dawley rats treated with daily repeated oral doses of MIL or OMPEP

Compound	Dose[a]		Toxic deaths/total
	mg kg^{-1} day^{-1}	μmol kg^{-1} day^{-1}	
MIL	46.4	113.8	5/6
	68.1	167.1	5/6
	100.0	245.3	6/6
OMPEP	68.1	143.2	0/6
	100.0	210.2	4/6
	147.0	309.0	6/6

[a] Treatment was conducted for 11 days; 6 animals were used in each treatment group

Statistical analysis. Student's *t*-test was used for statistical analysis of differences between the NBW changes registered in MIL- and OMPEP-treated groups, respectively.

Experiments in vitro. The soft-agar tumor stem-cell assay was performed according to Salmon et al. [13]. The test compound was added on day 0. Results were expressed as the concentration that reduced the number of colonies to 10% of the control value after 6 days of incubation (IC_{90} values). The following cell lines were used (the colony-forming efficiency is given in parentheses): L1210 (>95%), KB (>55%), and NK-lymphoma (>40%). Clonogenic microassays using agar-containing glass capillaries were performed as previously described in detail [9, 11]. Differentiation assays with HL-60 myeloid leukemic cells were carried out according to Maurer et al. [11].

Results

Acute oral toxicity

The oral LD_{50} for OMPEP in rats was found to be 631 mg/kg (1326.5 μmol/kg); in accordance with previous results, that for MIL was estimated to 296 mg/kg (726.2 μmol/kg).

Study of 11-day tolerability

The differences in the tolerability of the two alkylphospholipids after repeated oral administration are illustrated in Table 1. A dose of 68.1 mg/kg (143.2 μmol/kg) OMPEP could be given for 11 days running without being associated with mortality; the body weight loss was reversible, amounting to about 10 g (3.6%) at the end of treatment. In contrast, an extreme emaciation of more than 70 g (26%) was registered in the group treated with 46.4 mg/kg MIL (113.8 μmol/kg), and five of six animals died due to the treatment. A drop in food intake went hand in hand with the loss in body weight. In the group treated with 46.4 mg/kg MIL, complete anorexia developed, whereas in

Table 2. Vomiting induced in ferrets by single oral doses of MIL or OMPEP

Compound	Dose[a]		Number of animals vomiting/total	Mean time to onset of vomiting (min)
	mg/kg	μmol/kg		
MIL	30	73.6	1/6	210
	42.4	104.0	2/6	171
	60.0	147.2	6/6	74
OMPEP	45.0	94.6	0/6	–
	63.6	133.7	1/6	188
	90.0	189.2	3/6	132

[a] Each treatment group consisted of 6 animals

Table 3. Effect of MIL and OMPEP on the clonogenicity of three tumor cell lines in the soft-agar stem-cell assay

Cell line	$IC_{90}(\mu M)$[a]	
	MIL	OMPEP
L 1210	10.0±2.7	5.0±2.1
KB	7.6±3.2	0.6±0.1
NK-lymphoma	211 ±24	65, 67

[a] Mean values ± SD ($n = 3-6$); for $n = 2$, individual values are given

Table 4. Effect of MIL and OMPEP on the differentiation markers of HL-60 human promyelocytic leukemia cells cultured with various concentrations of inducing agent for 5 days

Alkyl-phospho-lipid	Concentration (μM)	Morphologically differentiated cells (%)[a]	Nitroblue tetrazolium reduction (%)[a]	Non-specific esterase-positive (%)[a]
MIL	0	4 ± 1	9 ± 4	4 ± 1
	1.0	12 ± 9	13 ± 6	7 ± 3
	2.5	14 ± 5	ND	ND
	5.0	17 ± 8	8 ± 3	14 ± 6
OMPEP	0	4 ± 1	9 ± 4	4 ± 1
	1.0	19 ± 9	29 ± 15	35 ± 1*
	2.5	49 ± 2*	ND	ND
	5.0	71 ± 6*	76 ± 10*	59 ± 2*

[a] Mean values ± SD for triplicate analyses; ND, not done
* Values significantly higher ($P < 0.05$, Student's t-test) than those obtained for MIL at the same concentration

rats given 68.1 mg/kg OMPEP the initially reduced food uptake had returned to nearly normal values by the end of the experiment. On postmortem examination, swelling of the intestinal wall and inflammation of the mucosa were found, particularly in MIL-treated animals.

Emetic potential of MIL and OMPEP in ferrets

The rate of vomiting and the latency period were found to be dose-dependent for both compounds. However, as shown in Table 2, the emetic potential of OMPEP was clearly lower than that of MIL.

Hematological observations

No hematological toxicity was registered in groups receiving various doses of OMPEP during the 21-day treatment or the additional 1-week observation period. In contrast to the significant increase observed in the total WBC in rats during daily treatment with MIL [5], even animals receiving the highest dose of OMPEP (46.4 mg/kg, corresponding to 97.5 μmol/kg) showed only a marginal elevation in WBC (results not shown).

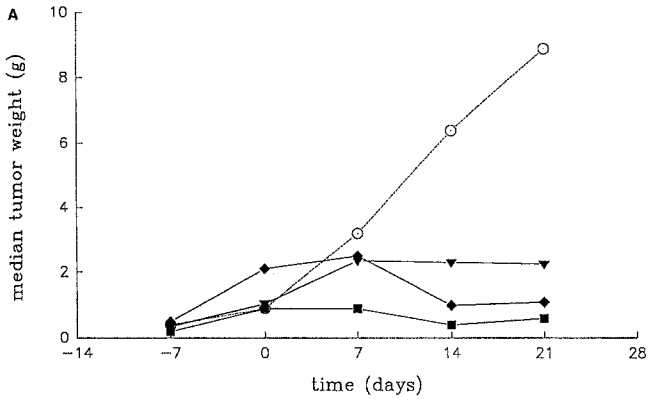
Antineoplastic activity in vitro

Table 3 displays the results of the comparative evaluation of OMPEP and MIL for in vitro activity against some cell lines. As can be seen, in all of the cell lines, OMPEP was more active than MIL, i.e., the IC_{90} value was lower. It is noteworthy that in the NK-lymphoma cell line, which is very resistant to MIL, OMPEP was about 3 times more active than the parent drug. The remarkable sensitivity of KB cells to OMPEP was confirmed by the result of the clonogenic microassay, which showed a 10-fold lower IC_{50} value for OMPEP (0.35 μM) than for MIL (3.5 μM). The differentiation-inducing effect of OMPEP in HL-60 human promyelocytic leukemia was concentration-related; at 5.0 μM OMPEP the percentage of morphologically differentiated cells (monocytes) was about 4 times higher than that observed at the same MIL concentration (Table 4). OMPEP induced differentiation even at a concentration as low as 1.0 μM, which did not inhibit the proliferation of HL-60 cells.

Antineoplastic activity in vivo

Small DMBA-induced tumors, daily doses. Both MIL and OMPEP induced regressions in only the groups receiving the two highest doses; in rats treated with 46.4 mg/kg (97.5 μmol/kg) OMPEP the tumors were hardly palpable at the end of the observation period (Fig 2); thus, the antineoplastic effect of this dose was slightly stronger than that of 31.6 mg/kg (77.5 μmol/kg) MIL (Table 5). Furthermore, there was a major difference with respect to the tolerability of the two compounds: treatment with 31.6 mg/kg MIL was associated with a substantial loss of body substance, whereas in rats given 46.4 mg/kg OMPEP a distinct gain in NBW was registered (Fig. 3). Similarly, 14.7 mg/kg (36.1 μmol/kg) MIL and 21.5 mg/kg (45.2 μmol/kg) OMPEP had about the same antineoplastic activity but exerted opposing effects on the NBW of treated animals (Table 5).

Large DMBA-induced tumors, daily doses. As can be seen in Fig. 4, for either the parent compound or its analogue a clear dose-response relationship was observed in mammary tumors weighing 4–6 g. Except at the lowest doses, marked remissions of tumors were attained. Under the conditions of this experiment, the better tolerability of OMPEP was again clearly demonstrated. The strong antineoplastic effect of 31.6 mg/kg MIL was achieved at the



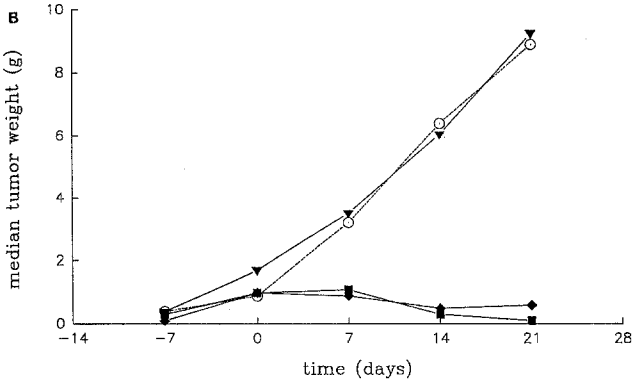


Fig. 2. (A) Antineoplastic action of a 14-day oral treatment (begun at t = 0) with various daily doses of MIL (▼, 6.81 mg/kg; ◆, 14.7 mg/kg; ■, 31.6 mg/kg; 0, tumor-bearing controls given 0.9% saline according to the same schedule) in Sprague-Dawley rats ($n = 6-7$) bearing small DMBA-induced mammary carcinomas. The median tumor weight is plotted versus the time in days before and after the start of the therapy. (B) Antineoplastic action of a 14-day oral treatment (begun at t = 0) with various daily doses of OMPEP (▼, 10.0 mg/kg; ◆, 21.5 mg/kg; ■, 46.4 mg/kg; 0, tumor-bearing controls given 0.9% saline according to the same schedule) in Sprague-Dawley rats ($n = 6-7$) bearing small DMBA-induced mammary carcinomas. The median tumor weight is plotted as a function of the time in days before and after the start of the therapy

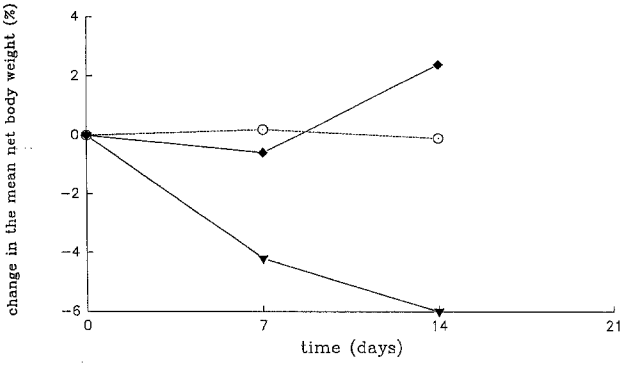


Fig. 3. Change in net body weight during a 14-day oral treatment of Sprague-Dawley rats ($n = 6-7$) bearing small DMBA-induced mammary carcinomas with 31.6 mg/kg MIL (▼) or 46.4 mg/kg OMPEP (◆). 0, tumor-bearing controls given 0.9% saline according to the same schedule. Mean deviations (%) from the net body weight registered at the start of the treatment (t = 0) are plotted versus the time in days on therapy

expense of a considerable loss in NBW, whereas in rats injected with an equieffective OMPEP dose of 46.4 mg/kg (Table 6), no decrease in body weight was registered at the end of the treatment (Fig. 5). Correspondingly, in the group treated with 21.5 mg/kg OMPEP the gain in NBW contrasted with the NBW loss observed in rats given 14.7 mg/kg MIL, exhibiting about the same antineoplastic activity (Table 6).

Large DMBA-induced tumors, high single doses. MIL at a dose of 237 mg/kg (581.4 μmol/kg) induced rapid regressions of tumors to about one-third of the value registered at the time of injection (Fig. 6A). In the groups receiving the two lower doses the antineoplastic effect was distinctly weaker; surprisingly, 110 mg/kg (269.9 μmol/kg) was more effective than 162 mg/kg (397.4 μmol/kg); (Fig. 6A). The tumors of all MIL-treated animals resumed growing 7 days after the dosing (Fig. 6A). Figure 6B illustrates the dose dependency of the antitumor action of

Table 5. Antineoplastic effects and changes in net body weight observed in rats bearing small DMBA-induced mammary carcinomas following a 14-day oral treatment with various daily doses of MIL or OMPEP

Compound	Dose		$\frac{T}{C} \times 100\%$[a]		$\frac{m_t}{m_o} \times 100\%$[b]		BWD (%)[c]
	mg/kg	μmol/kg	14 days	21 days	14 days	21 days	
Controls	0	0	–	–	693.9	908.2	–0.04
MIL	6.81	16.7	33.8	25.3	219.0	214.3	–1.6
	14.7	36.1	14.7	12.4	47.6	52.4	–3.6
	31.6	77.5	5.9	6.7	44.4	66.7	–6.1
OMPEP	10.0	21.0	88.9	104.5	355.9	547.1	–0.12
	21.5	45.2	7.4	6.7	50.0	60.0	+2.6*
	46.4	97.5	4.4	1.1	30.0	10.0	+2.5**

[a] $\frac{T}{C} \times 100\%$ = quotient of the median tumor mass of the treated and control groups x 100, evaluated at a specified day after the start of the treatment

[b] $\frac{m_t}{m_o} \times 100\%$ = quotient of the median tumor mass at a specified day of the treatment and at the start of administration (t = o), respectively, $\times 100$

[c] Mean net body-weight difference (end of the treatment – start of the treatment) expressed as a percentage of the value at t = 0

* $P < 0.05$ vs 14.7 mg/kg MIL; **$P < 0.05$ vs 31.6 mg/kg MIL

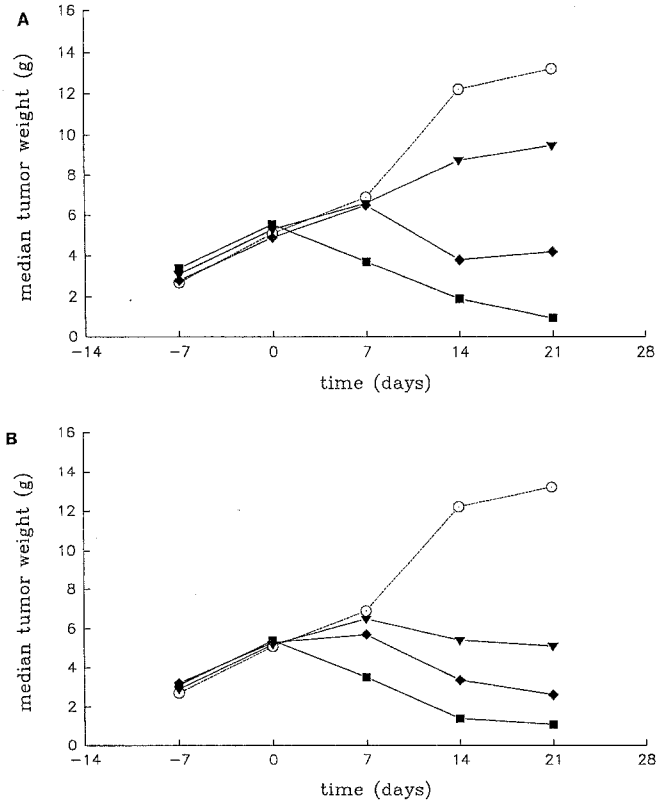


Fig. 4. (A) Antineoplastic effect of a 14-day oral treatment (begun at t = 0) with various daily doses of MIL (▼), 6.81 mg/kg; ◆, 14.7 mg/kg; ■, 31.6 mg/kg; 0, tumor-bearing controls given 0.9% saline according to the same schedule) in Sprague-Dawley rats ($n = 6-7$) bearing large DMBA-induced mammary carcinomas. The median tumor weight is plotted as a function of the time in days before and after the start of the therapy. (B) Antineoplastic effect of a 14-day oral treatment (begun at t = 0) with various daily doses of OMPEP (▼, 10.0 mg/kg; ◆, 21.5 mg/kg; ■, 46.4 mg/kg; 0, controls given 0.9% saline according to the same schedule) in Sprague-Dawley rats ($n = 6-7$) bearing large DMBA-induced mammary carcinomas. The median tumor weight is plotted versus the time in days before and after the start of the therapy

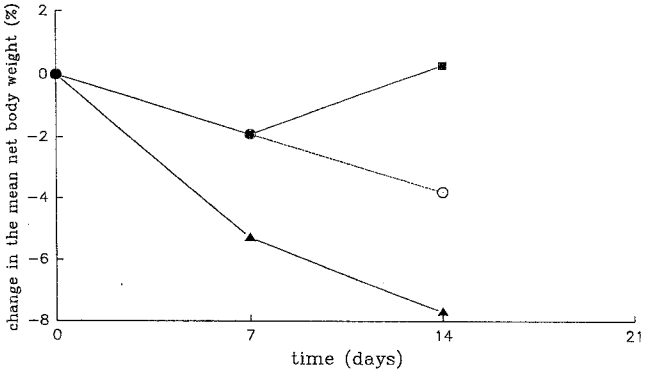


Fig. 5. Change in net body weight during a 14-day oral treatment of Sprague-Dawley rats ($n = 6-7$) bearing large DMBA-induced mammary carcinomas with 31.6 mg/kg MIL (▲) or 46.4 mg/kg OMPEP (■). 0, tumor-bearing controls given 0.9% saline according to the same schedule. Mean deviations (%) from the net body weight registered at the start of the treatment (t = 0) are plotted versus the time in days on therapy

OMPEP. At all three doses regressions of the neoplasms were attained, but only the tumors treated with 511 mg/kg (1074.2 μmol/kg) did not start to regrow during the 14-day observation period following the injection.

After the injection a rapid decrease in NBW was seen in all rats. With the exception of the groups receiving the highest doses (237 mg/kg MIL and 511 mg/kg OMPEP), it began to rise 3 days following the treatment. At the end of the experiment, the NBW of all injected animals was considerably higher than that of controls (Table 7). However, since 237 mg/kg MIL represents the approximate LD_{10} one of seven rats in this group died 3 days after the treatment. In contrast, 348 mg/kg (731.6 μmol/kg) OMPEP, although exhibiting the same antitumor activity as 237 mg/kg MIL (Table 7), lay far outside the lethal portion of the toxic dose-response curve; besides, the recovery from the mean nadir of body weight commenced earlier and the increase in NBW was twice as high as that observed in the corresponding MIL-treated group at the end of the experiment

Table 6. Antineoplastic effects and changes in net body weight observed following a 14-day oral treatment of rats bearing large DMBA-induced mammary carcinomas with various daily doses of MIL or OMPEP

Compound	Dose		$\frac{T}{C} \times 100\%$[a]		$\frac{m_t}{m_o} \times 100\%$[b]		BWD (%)[c]
	mg/kg	μmol/kg	14 days	21 days	14 days	21 days	
Controls	0	0	–	–	239	258.8	–3.8
MIL	6.81	16.7	71.7	72.0	165.1	179.2	–5.4
	14.7	36.1	30.3	31.3	70.0	75.0	–2.9
	31.6	77.5	15.6	7.2	34.0	17.1	–7.6
OMPEP	10.0	21.0	44.3	38.6	104.0	98.1	–1.6
	21.5	45.2	27.5	19.7	63.0	49.1	+1.7*
	46.4	97.5	11.5	8.4	26.0	20.3	+0.3**

[a] $\frac{T}{C} \times 100\%$ = quotient of the median tumor mass of the treated and control groups $\times 100$, evaluated at a specified day after the start of the treatment

[b] $\frac{m_t}{m_o} \times 100\%$ = quotient of the median tumor mass at a specified day of the treatment and at the start of administration (t = 0), respectively, $\times 100$

[c] Mean net body-weight difference (end of the treatment – start of the treatment) expressed as a percentage of the value at t = 0

* $P = 0.05$ vs 14.7 mg/kg MIL; **$P < 0.05$ vs 31.6 mg/kg MIL

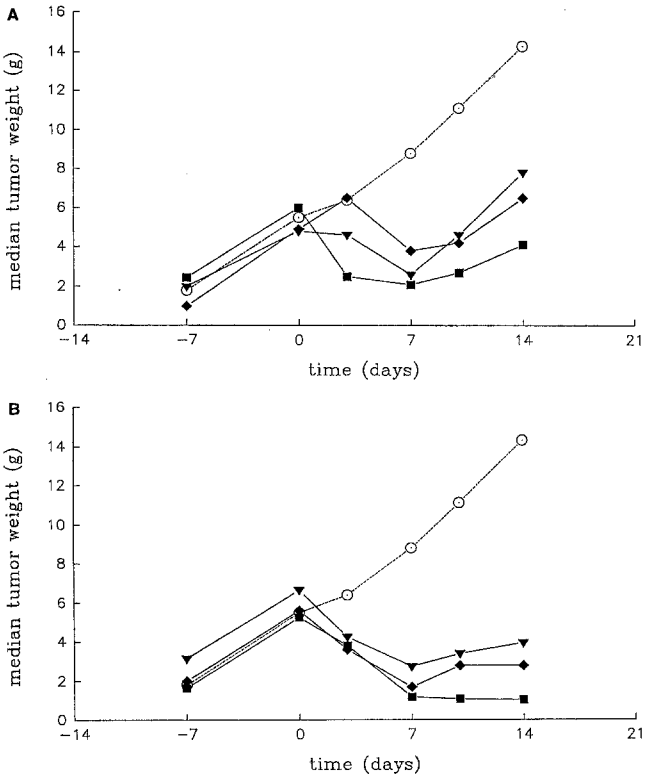


Fig. 6. (A) Antineoplastic activity of high single oral doses of MIL given at t = 0 (▼, 110 mg/kg; ◆, 162 mg/kg; ■, 237 mg/kg; 0, tumor-bearing controls given 0.9% saline) in Sprague-Dawley rats ($n = 6-7$) bearing large DMBA-induced mammary carcinomas. The median tumor weight is plotted versus the time in days before and after the MIL injection. (B) Antineoplastic activity of high single oral doses of OMPEP given at t = 0 (▼, 237 mg/kg; ◆, 348 mg/kg; ■, 511 mg/kg; 0, tumor-bearing controls given 0.9% saline) in Sprague-Dawley rats ($n = 6-7$) bearing large DMBA-induced mammary carcinomas. The median tumor weight is plotted versus the time in days before and after the OMPEP injection

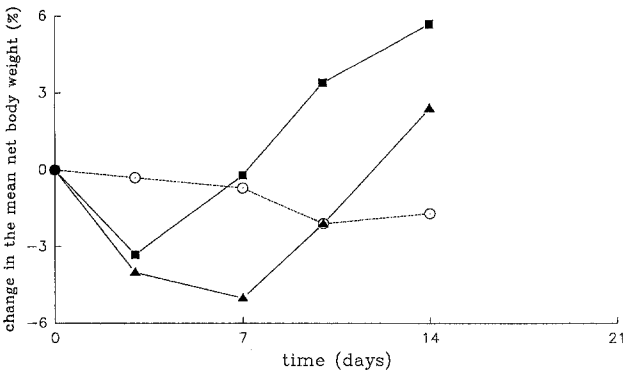


Fig. 7. Change in net body weight (following the oral administration (at t = 0) of a single dose of MIL (▲, 237 mg/kg; $n = 7$) or OMPEP (■, 348 mg/kg; $n = 7$) in Sprague-Dawley rats bearing large DMBA-induced mammary carcinomas. 0, tumor-bearing controls ($n = 7$) given 0.9% saline only. Mean deviations (%) from the net body weight registered at t = 0 are plotted versus the time in days after the treatment

(Fig. 7). Even in the group treated with the highest OMPEP dose (511 mg/kg), none of the rats died; moreover, the therapeutic effect was greater that than seen at any other single dose of the two alkylphospholipids (Table 7).

Discussion

The toxic effects of oral MIL in rats primarily involve the gastrointestinal tract [12]. Macroscopical and microscopical examination of treated animals revealed, above all, severe enterocolitis, accounting for the diarrhea and weight loss. Correspondingly, in clinical phase I studies of oral MIL, the dose-limiting gastrointestinal side effects were nausea, vomiting, and diarrhea [3]. In an attempt to increase the therapeutic range, new analogues of MIL have been synthesized, assuming that the replacement of choline by a suitable saturated ring containing a tetracoordinate nitrogen atom would lead to alkylphospholipids less toxic than the parent compound without substantially reducing the antineoplastic potency. With the substitution of *N*-methylpiperidine for choline in the hydrophilic portion and that of an octadecyl group for the hexadecyl group in the apolar region of the molecule, these suppositions have been met to a large extent. The acute oral LD_{50} of OMPEP

Table 7. Antineoplastic effects and changes in net body weight observed in rats with large DMBA-induced mammary carcinomas following the administration of high single doses of MIL or OMPEP

Compound	Dose		$\frac{T}{C} \times 100\%$[a]	$\frac{m_7}{m_o} \times 100\%$[b]	BWD (%)[c]
	mg/kg	μmol/kg			
Controls	0	0	–	160	−1.7
MIL	110	269.9	29.5	54.0	+0.3
	162	397.4	43.2	78.0	+2.6
	237	581.4	23.9	35.0	+2.4
OMPEP	237	498.2	31.3	41.0	+4.8
	348	731.6	19.3	30.0	+5.7*
	511	1074.2	13.6	23.0	+1.8

[a] $\frac{T}{C} \times 100\%$ = quotient of the median tumor mass of the treated and control groups $\times 100$ (evaluated at day 7 after dosing)

[b] $\frac{m_7}{m_o} \times 100\%$ = quotient of the median tumor mass at t = 7 days and at the day of injection (t = 0), respectively, $\times 100$

[c] Mean net body-weight difference (14 days after dosing – the day of treatment) expressed as a percentage of the value at t = 0

* $P = 0.05$ vs 237 mg/kg MIL

in rats was twice as high as that of MIL. In the 11-day tolerability study, 46.4 mg/kg MIL proved to be clearly more toxic than the 1.5-fold higher dose of 68.1 mg/kg OMPEP. Furthermore, the emetic potential of OMPEP in ferrets was distinctly lower than that of MIL. In vitro, OMPEP turned out to be superior to MIL in several cell lines. Generally, to attain the same antineoplastic effect in DMBA-induced tumors, the OMPEP doses had to be 1.5 times higher than those of MIL. However, treatment with such equieffective daily doses of the novel analogue was substantially better tolerated than that with the parent compound, as evidenced by the gain in NBW noted following therapy with appropriate OMPEP doses and the loss of body substance registered in the corresponding MIL groups. Furthermore, the highest dose of MIL given in this study (1×237 mg/kg) was associated with mortality, whereas an OMPEP dose as high as 1×511 mg/kg not only exerted a stronger antitumor activity than could be achieved by MIL but was also free of lethal effects.

It follows from these experiments that choline cannot be regarded as an essential constituent of chemotherapeutically active alkylphospholipid molecules. Moreover, the therapeutic range of the *N*-methylpiperidine analogue of MIL proved to be broader than that of the parent compound in rats bearing DMBA-induced tumors. Consequently, the toxic and antineoplastic properties of alkylphospholipids can be separated to some extent, which implies that the mechanism of their antineoplastic action may be different from that responsible for their toxicity. Taken together, our findings support the introductory hypothesis assuming a causal connection between the toxicity of alkylphospholipids and the parasympathomimetic activity of the nitrogenous base forming their hydrophilic heads. In this context, it may be of significance that methylpiperidinium analogues of acetylcholine exert a much weaker parasympathomimetic activity than acetylcholine itself [1].

The lower toxicity of OMPEP as compared with MIL is even more noteworthy, since octadecylphosphocholine, despite its containing the same alkyl group as OMPEP, turned out to be distinctly more toxic than MIL [2]. Thus, the observation that OMPEP is better tolerated than both C_{16}- and C_{18}-phosphocholine must be ascribed solely to the *N*-methylpiperidine forming the polar region of the novel analogue and not to the substitution of the octadecyl group for the hexadecyl group in the hydrophobic portion of the molecule. Furthermore, again in contrast to the results obtained with the two alkylphosphocholines, OMPEP was less toxic than its hexadecyl homologue (Stekar, unpublished results), i.e., the dependence of the toxicity on the length of the alkyl group was inverted when choline was replaced by *N*-methylpiperidine. Similarly, hexadecyl and oleyl analogues of OMPEP turned out to be inactive or only weakly active in DMBA-induced tumors (Stekar, unpublished results), which contrasts with the finding that in this tumor model, oleylphosphocholine had strong antineoplastic activity comparable with that of MIL (Eibl and Berger, personal communication; Stekar, unpublished results). Thus, the substitution of *N*-methylpiperidine for choline led to alkylphospholipids whose antineoplastic and toxic properties differed in an unexpected way from those of alkylphosphocholines. The new head group modulated the dependence of the biological effects on the length of the alkyl chain and its degree of saturation in its own specific way, which could not be anticipated on the basis of results obtained in the series of alkylphosphocholines. These unpredictable findings could indicate that both the toxic and the antineoplastic effects of alkylphospholipids may at least in part be due to the intact molecule and not to some degradation products; a concerted action of the hydrophilic and hydrophobic regions of the OMPEP molecule on some putative target structure may be required for a marked antineoplastic effect.

In all experimental groups a characteristic pattern for the change in NBW was observed. In control animals the registered decrease in body mass was directly proportional to the tumor size, i.e., the larger the tumor, the higher its toxicity for the host. This is a clinically well-known yet unexplained phenomenon. The NBW change observed in OMPEP-treated animals was probably the result of two opposing effects. On the one hand, by virtue of its antineoplastic activity the heterocyclic alkylphospholipid presumably reduced the toxicity of the tumor for the host, thus counteracting or even neutralizing its negative influence on NBW. On the other hand, the toxicity of OMPEP or MIL may have added to that of the tumor, thus intensifying NBW loss. Depending on the dose and the size of the tumor, the result of these two opposite effects should be different. This assumption would account for the observation that in rats injected with the lowest daily dose of OMPEP a loss in NBW was observed, whereas treatment with the two higher doses (small tumors) or the intermediate dose (large tumors) resulted in an increase in NBW at the end of the treatment. The same explanation might also apply for the experiments with high single OMPEP doses and for the observation that in MIL-treated animals bearing large tumors, the smallest loss in NBW was observed in the group injected with the intermediate daily dose.

The mode of action of alkylphospholipids remains a matter of conjecture (for a review see [7]). Additionally, two recent aspects must be considered in discussions of this topic. The first involves the lack of cytotoxicity observed at concentrations that induce differentiation, indicating programmed cell death (apoptosis) as a factor in the antineoplastic action of alkylphospholipids (Maurer et al., manuscript in preparation). The second aspect is the high sensitivity of KB tumors [15] and DMBA-induced breast carcinomas to alkylphospholipids, which goes hand in hand with high levels of epidermal growth factor (EGF) receptors on the cell membrane of these neoplasms [10]; this finding suggests that the antineoplastic action of alkylphospholipids might at least partly be due to their interference with EGF binding to receptors [8]. On the basis of the high antineoplastic activity as well as the considerably better tolerability of OMPEP in comparison with MIL, clinical trials of this interesting new antitumor agent will be started in the near future.

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