# Use of alkylphosphocholines in combination with antitumor medicaments

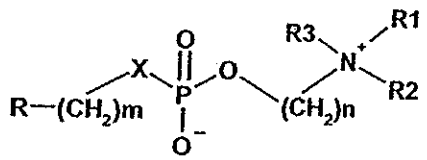
Alkylphosphocholines are a new class of organic compounds which show diverse antineoplastic activities (M. Lohmeyer and R. Bittman; Antitumor ether lipids and alkylphosphocholines, DOF, **19** (11), 1021-1037 (1994)). The effect of the alkylphosphocholines in this connection may be based on various molecular and biochemical mechanisms, some of which take place at the level of the plasma membrane of the cells. It is well known that alkylphosphocholines influence inositol metabolism, the interaction with phospholipases or inhibition of protein kinase C and thus that this class of substances has a general influence on cellular signal transduction (K. Maly, F. Überall, C. Schubert, E. Kindler, J. Stekar, H. Brachwitz and H. H. Grunicke, Interference of new alkylphospholipid analogues with mitogenic signal transduction, Anti-Cancer Drug Design, **10**, 411-425 (1995)). Thus, the alkylphosphocholine perifosine shows growth-inhibitory properties in relation to various melanoma, CNS, lung, colon, prostate and breast cancer cell lines with an $IC_{50}$ in the region of 0.2 – 20 μM (P. Hilgard, T. Klenner, J. Stekar, G. Nössner, B. Kutscher and J. Engel; D-21266, a New Heterocyclic Alkylphospholipid with Antitumor Activity, Eur. J. Cancer, **33** (3), 442-446 (1997)). It is further known that perifosine blocks tumor cells in the $G_1$-S and $G_2$-M phase of the cell cycle (V. Patel, T. Lahusen, T. Sy, E. A. Sausville, J. S. Gutkind and A. M. Senderowicz; Perifosine, a Novel Alkylphospholipid, Induces $p21^{Waf1}$ Expression in Squamous Carcinoma Cells through a p53-independent Pathway, Leading to Loss in Cyclin-dependent Kinase Activity and Cell Cycle Arrest, Cancer Research **62**, 1401-1409 (2002)).

It is known that the use of alkylphosphocholines before or together with radiotherapy leads to synergistic effects in the treatment of tumors (G.A. Ruitter, M. Verheijl, S.F. Zerp and W.J. van Blitterswijk; Alkyl-Lysophospholipids as Anticancer Agents and Enhancers of Radiation-Induced Apoptosis, Int. J. Radiation Oncology Biol. Phys., **49** (2), 415-420, 2001). It has also been reported that various glycero-3-phospholipids, e.g. ET-18-$OCH_3$, in combination with various DNA-interacting substances or tubulin binders increase the antitumor activity in vitro on various tumor cell lines (A. Noseda, M.E. Berens, J.G. White and E.J. Modest; In vitro antiproliferative activity of combinations of ether lipid analogs and DNA-Interactive

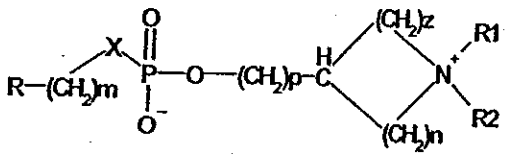
agents against human tumor cells, Cancer Res., 48 (7), 1788-1791 (1988); P. Principe, H. Coulomb, C. Broquet and P. Braquet; Evaluation of combinations of antineoplastic ether phospholipids and chemotherapeutic drugs, Ant-Cancer Drugs, 3 (6), 577-587 (1992); P. Principe, H. Coulomb, J.-M. Mencia-Huerta, C. Broquet and P. Braquet; Synergistic cytotoxic effect of aza-alkylphospholipids in association with chemotherapeutic drugs, J. Lipid Mediators Cell Signalling, 10 (1-2), 171-173 (1994)).

It has now been possible, surprisingly, to show that linear alkylphosphocholines of the general formula I and II are suitable for use in a combination according to the invention with other drug products for the treatment of benign and malignant oncoses in humans and mammals.

Accordingly, the present invention provides the use of at least one alkylphosphocholine of the general formula I and II




formula I




formula II

wherein, independently of one another:

n, m, p, z : are an integer between 0 and 4;

X are O, S, NH;

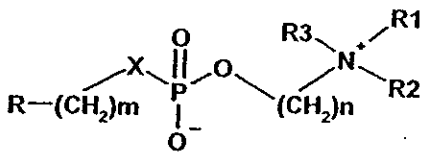
R is H, a straight-chain or branched ($C_1$-$C_{20}$)-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals;

$R_1$, $R_2$, $R_3$ is, independently of one another, H, a straight-chain or branched ($C_1$-$C_6$)-alkyl radical, a ($C_3$-$C_7$)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals, for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament, selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine.

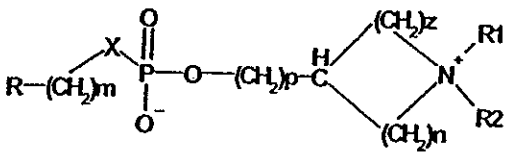
Antitumor substances may be alkylating agents, antimetabolites, plant alkaloids, platinum compounds, tumor antibiotics and agonists or antagonists of natural hormones, and specially are selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluorouracil, fludarabine and cytarabine.

The alkylphosphocholines of the general formula I and II on which the invention is based can be used in the form of finished drug products.

The compounds on which the invention is based are described by the general formulae I and II:



formula I



formula II

where, independently of one another:

n, m, p, z are an integer between 0 and 4;

*X* is O, S, NH;

R is H, a straight-chain or branched ($C_1$-$C_{20}$)-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals.

$R_1$, $R_2$, $R_3$ is, independently of one another, H, a straight-chain or branched ($C_1$-$C_6$)-alkyl radical, preferably methyl and ethyl, a ($C_3$-$C_7$)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals.

The medicament is suitable for use for controlling tumors in humans and in mammals.

The therapeutically effective dose, suitable for being administered for the treatment, of the particular compound of the general formula I and II on which the invention is based depends inter alia on the nature and the stage of the oncosis, the age and sex of the patient, the mode of administration and the duration of treatment.

The compounds on which the invention is based can be administered in a drug product as liquid, semisolid and solid drug forms. This takes place in the manner suitable in each case in the form of aerosols, oral powders, dusting powders and epipastics, uncoated tablets, coated tablets, emulsions, foams, solutions, suspensions, gels, ointments, pastes, pills, pastilles, capsules or suppositories.

**Exemplary embodiments:**

1. Administration of perifosine (D-21 266) in combination with cisplatin

Although the combination of this Example falls outside the scope of the claims of this application, the Example helps to illustrate the invention and is therefore retained for clarity and completeness.

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.
Cisplatin 4 x1 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

| Treatment | Tumor Initial weight [g] | Day 21 Change in [%] | p test vs. Control |
|---|---|---|---|
| Control | 1.0 | 875 | - |
| Perifosine (D-21266) | 0.9 | -25 | <0.001 |
| Cisplatin | 0.9 | 410 | 0.120 |
| Perifosine (D-21266) + Cisplatin | 0.8 | -75 | <0.001 |

2. Administration of perifosine in combination with cyclophosphamide

Although the combination of this Example falls outside the scope of the claims of this application, the Example helps to illustrate the invention and is therefore retained for clarity and completeness.

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

Administration: Perifosine 14 x 6.81 mg/kg p.o.
Cyclophosphamide 100 mg/kg, VZ 0, i.v.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

| Treatment | Tumor Initial weight [g] | Day 21 Change in [%] | p test vs. Control |
|---|---|---|---|
| Control | 1.0 | 875 | - |
| Perifosine (D-21266) | 0.9 | -25 | <0.001 |
| Cyclophosphamide | 0.9 | 500 | 0.011 |
| Perifosine (D-21266) + Cyclophosphamide | 0.8 | -83.3 | <0.001 |

3. Administration of perifosine in combination with doxorubicin (Adriamycin)

In vivo test: DMBA-induced rat mammary carcinoma model

Experimental animal: Sprague-Dawley rat, female

Procedure: The mammary carcinoma was induced by a single oral dose of DMBA. The animals received perifosine from day 0 to day 14 and were observed up to day 42. The weight of the tumor was mass was estimated by palpation and comparison with plastic models. The initial weight is set equal to 100%.

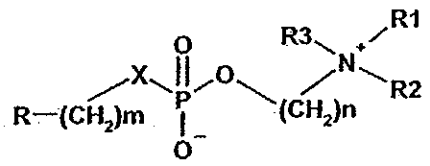
Administration: Perifosine 14 x 6.81 mg/kg p.o.
Adriamycin 4 x 2.15 mg/kg i.p.

Effect: Reduction in the tumor was distinctly greater and longer through the combination treatment than through the single treatment in each case.

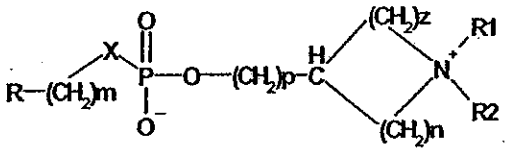
| Treatment | Tumor Initial weight [g] | Tag 21 Change in [%] | p test vs. Control |
|---|---|---|---|
| Control | 1.0 | 875 | - |
| Perifosine (D-21266) | 0.9 | -25 | <0.001 |
| Adriamycin | 1.0 | 781.3 | 0.197 |
| Perifosine (D-21266) + Adriamycin | 01.0 | -70 | <0.001 |

**Claims**

1. The use of at least one alkylphosphocholine of the general formula I and II



formula I



formula II

wherein, independently of one another:

n, m, p, z are an integer between 0 and 4;

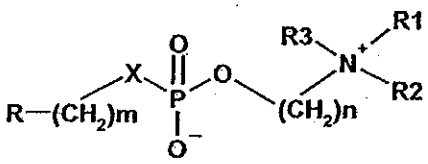
X are O, S, NH;

R is H, a straight-chain or branched ($C_1$-$C_{20}$)-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals;

$R_1$, $R_2$, $R_3$ is, independently of one another, H, a straight-chain or branched ($C_1$-$C_6$)-alkyl radical, a ($C_3$-$C_7$)-cycloalkyl radical and which may be unsubstituted or optionally substituted on the same or on different C atoms by one, two or more halogen, nitro, cyano, hydroxyl, ($C_1$-$C_6$)-alkoxy, amino, mono-($C_1$-$C_4$)-alkylamino or di-($C_1$-$C_4$)-alkylamino radicals,

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament, selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine.

2. The use of an alkylphosphocholine of the general formula I as claimed in claim 1




formula I

wherein, independently of one another:

n is the integer 1 or 2;

m is the integer 1;

X is O;

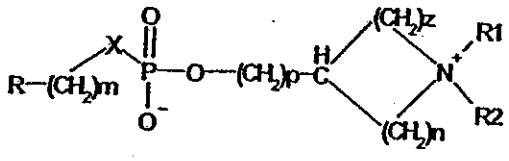
R is H or a straight-chain or branched ($C_1$-$C_{17}$)-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds;

$R_1$, $R_2$, $R_3$ is, independently of one another, H, a straight-chain or branched ($C_1$-$C_6$)-alkyl radical, or a ($C_3$-$C_7$)-cycloalkyl radical;

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament, selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin

(Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine.

3. The use of an alkylphosphocholine of the general formula II as claimed in claim 1



formula II

wherein, independently of one another:

m, p is the integer 1;

n, z is the integer 2;

X is O;

R is H, a straight-chain or branched ($C_1$-$C_{17}$)-alkyl radical which may be saturated or unsaturated with one to three double and/or triple bonds;

$R_1$, $R_2$, $R_3$ is, independently of one another, H, a straight-chain or branched ($C_1$-$C_6$)-alkyl radical, or a ($C_3$-$C_7$)-cycloalkyl radical;

for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine.

4. The use as claimed in any of claims 1-3 wherein the ($C_1$-$C_6$)-alkyl radical is methyl or ethyl.

5. The use of octadecyl 1,1-dimethylpiperidinio-4-yl phosphate as claimed in claim 1 for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament, selected from the group consisting of carboplatin, oxaliplatin, bleomycin,

doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine.

6. The use as claimed in any of claims 1-5, wherein the antitumor medicament is doxorubicin (Adriamycin).

7. The use of alkylphosphocholines of the general formula I and II as claimed in claims 1 to 6, where the antitumor medicament is a combination of various cytostatics.

8. The use of at least one alkylphosphocholine of the formula I and II as claimed in claims 1 to 4 for the manufacture of a drug product for the treatment of benign and malignant oncoses before and/or during treatment with an antitumor medicament, selected from the group consisting of carboplatin, oxaliplatin, bleomycin, doxorubicin (Adriamycin), methotrexate, paclitaxel, docetaxel, vincristine, vinblastine, etoposide, teniposide, ifosfamide, 5-fluoruracil, fludarabine and cytarabine, wherein the drug product comprises the customary pharmaceutical carriers, excipients and/or diluents in addition to the at least one alkylphosphocholine of the formula I and II.