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Phase I and pharmacological study of daily oral administration of perifosine (D-21266) in patients with advanced solid tumours

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Abstract

Alkylphosphocholines are a novel class of antitumour agents structurally related to ether lipids that interact with the cell membrane and influence intracellular growth signal transduction pathways. We performed a phase I trial with an analogue of miltefosine, perifosine (D-21266), which was expected to induce less gastrointestinal toxicity. Objectives of the trial were: to determine the maximum-tolerated dose (MTD) for daily administration, to identify the dose-limiting toxicity (DLT) of this schedule, to assess drug accumulation and to determine the relevant pharmacokinetic parameters. 22 patients with advanced solid tumours were treated at doses ranging from 50 to 350 mg/day for 3 weeks, followed by 1 week of rest. Toxicity consisted mainly of gastrointestinal side-effects: nausea was reported by 11 patients (52%, 10 patients Common Toxicity Criteria (CTC) grades 1–2 and 1 patient CTC grade 3), vomiting by 8 (38%, all CTC grades 1–2), and diarrhoea by 9 (43%, 8 patients CTC grades 1–2 and 1 patient CTC grade 3). The severity of these side effects appeared to increase with increasing doses. Another common side-effect was fatigue, occurring in 9 patients (43%). No haematology toxicity was observed. Dose-limiting toxicity (DLT) was not reached, but gastrointestinal complaints led to an early treatment discontinuation in an increasing number of patients at the higher dose levels. Therefore, MTD was established at 200 mg/day. The pharmacokinetic studies suggested dose proportionality.

Keywords: Perifosine; Ether lipids; Phase I trial

1. Introduction

Synthetic, membrane-permeable ether lipids have been studied as antitumour agents for over 20 years. There are two classes of ether lipids: alkyllysophospholipids and alkylphosphocholines. They both show antineoplastic activity *in vitro* and *in vivo* [1–3], by activating cytotoxic macrophages, by inhibiting neoplastic cell invasion in normal tissue [4–6], and by inducing apoptosis [7–9]. Ether lipids act primarily at the cell membrane: because of their resistance to phospholipase, they can accumulate in the cell and other membranes [10,11], where they influence signal transduction by interfering with the mitogen-activated protein kinase (MAPK) pathway. Although the exact mechanism of action has not been fully elucidated, several observed effects provide clues to the nature of the antineoplastic activity of the ether lipids: they interfere with the metabolism of phospholipid constituents of the cell membrane [12,13], they reduce phospholipase C-mediated inositol 1,4,5-triphosphate formation and calcium release [14–16] and they inhibit protein kinase C [17–19]. The induction of apoptosis is probably a result of multiple effects: firstly, inhibition of MAPK activation combined with stimulation of the SAPK/JNK pathway [9]. The balance between these pathways is a determinant of the tendency of a cell to undergo apoptosis [20]. Secondly, induction of myc-expression, another effector

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of apoptosis [8] and thirdly, an increase of the cytosolic calcium level, which also triggers a pro-apoptotic pathway [16].

Miltefosine was the first alkylphosphocholine to be evaluated in clinical trials. Because miltefosine, and all other ether lipids, induce haemolysis, parenteral administration is not possible. Oral administration of miltefosine resulted in severe gastrointestinal toxicity, in particular nausea, anorexia, vomiting and diarrhoea [21,22]. Topical formulations, however, have become a valuable tool in the management of cutaneous metastases of breast cancer and, occasionally, other solid tumours [23–25]. Analogue research to produce compounds with a better systemic therapeutic index than miltefosine yielded D-21266 (octadecyl-(1,1-dimethyl-4-piperidylio)phosphate) or perifosine (Fig. 1). In D-21266, the choline head group has been substituted by a cyclic aliphatic piperidyl residue. The major metabolite of miltefosine, phosphocholine, has a structure resembling that of acetylcholine [26], which may be the reason for the severe gastrointestinal disturbances observed upon oral treatment. Perifosine is not able to generate phosphocholine, and hence may be better tolerated [27].

In vitro, perifosine showed antineoplastic effects against melanoma, nervous system, lung, prostate, colon and breast cancers, with an activity similar to or stronger than that of miltefosine [28]. Furthermore, in human leukaemia cells, perifosine increased the rate of apoptosis in a dose-dependent manner. This effect was even stronger in combination with radiation, suggesting a favourable profile of perifosine in combination therapies [9]. *In vivo* preclinical studies have been performed on various animal tumour models, including both syngeneic murine tumours and human xenografts. High antineoplastic effects were observed, which could be enhanced by introducing a dose schedule consisting of a high loading dose followed by a lower maintenance dose [28]. Preclinical pharmacokinetic investigations showed a high oral bioavailability and a long terminal half-life of perifosine in rats (Asta Medica, data not shown).

Here, we present the results of a phase I clinical and pharmacokinetic trial of daily oral administration of perifosine in patients with advanced solid tumours. The objectives of the trial were: (a) to determine the maximum-tolerated dose (MTD) for daily administration of perifosine, (b) to identify the dose-limiting toxicity (DLT) and (c) to assess drug accumulation and, if possible, the terminal half-life.

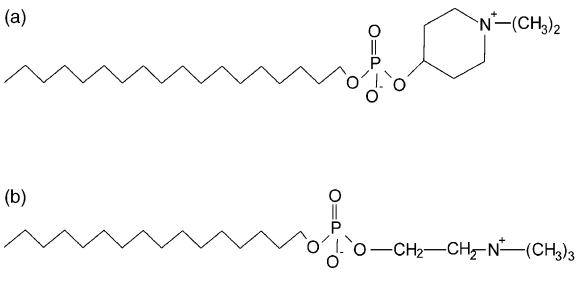

Fig. 1. Structural formulas of perifosine (a) and miltefosine (b).

2. Patients and methods

2.1. Patient population

Patients were eligible if they had a histologically-confirmed diagnosis of a solid malignant tumour not amenable to standard therapy. Other eligibility criteria included an European Cooperative Oncology Group-World Health Organization (ECOG-WHO) performance status $\leqslant 2$, anticipated life expectancy of $\geqslant 3$ months and age $\geqslant 18$ years. Previous anti-cancer immuno-, chemo- or radiotherapy had to be discontinued for at least 4 weeks before entry into the study, or 6 weeks in cases of pretreatment with nitrosoureas or mitomycin C. All patients had to have acceptable bone marrow function, defined by white blood cells (WBC) $\geqslant 4\times10^9$ cells/l, platelets $\geqslant 100\times10^9$ cells/l and haemoglobin $\geqslant 6.8$ mmol/l (11 g/dl); serum bilirubin $\leqslant 25$ μmol/l (1.5 mg/dl), aminotransferases $\leqslant$ twice the normal upper limit or $\leqslant$ five times when related to liver metastases, and serum creatinine $\leqslant 135$ μmol/l (1.5 mg/dl). Exclusion criteria consisted of concomitant or recent (within 4 weeks) treatment with other investigational drugs, history of haemolytic events, any condition classified as common toxicity criteria (CTC, version 21-12-1994) grade >1 (except if caused by the underlying malignant disease—in this case, CTC grade $\leqslant 2$ was allowed), brain metastases or leptomeningeal disease, recent acute or chronic gastrointestinal conditions that might predispose for intolerability or poor drug absorption, any non-compensated or uncontrolled non-malignant condition, pre-existing retinal disease or pathological baseline electro-oculogram and breast feeding, pregnancy or inadequate contraception in women of childbearing potential and men. The study protocol was approved by the Medical Ethics Committee of the Institute, and all patients gave written informed consent prior to the study.

2.2. Treatment plan and study design

D-21266 or perifosine was supplied by ASTA Medica AG (Frankfurt/Main, Germany) as a 50-mg film-coated tablet, soluble in gastric juice. The starting dose-level was 50 mg/day for 3 weeks, followed by 1 week of a treatment-free period each cycle. This dose was based on the finding that 350 mg once weekly was tolerated in humans [29]. An interpatient dose escalation scheme was used. Initially, 3 patients were recruited at each dose-level, and 3 more were entered in cases of significant toxicity (defined as haematological toxicity

$\geqslant$CTC grade 3 or non-haematological toxicity $\geqslant$CTC grade 2 despite prophylactic treatment). The full dose was taken at once, together with a meal. Patients were scheduled to receive at least two courses. Patients with progressive disease were removed from the study.

2.3. Patient evaluation

Pretreatment evaluation included a complete medical history and complete physical examination. Indicator lesions were measured before start of treatment and repeatedly during the study, as a basis for the assessment of efficacy of perifosine. Before each course, blood chemistry and urine were checked. During cycle 1, these assessments were made twice weekly, and during the rest of treatment once weekly. All toxicities observed were graded according to the CTC [30]. DLT was defined as an adverse event which is likely related to the study treatment with an intensity of CTC grade $\geqslant$3 (non-haematological toxicity) or 4 (haematological toxicity), despite symptomatic/prophylactic treatment. The MTD was defined as the dose level where 2 or more out of 6 patients experienced a DLT.

2.4. Pharmacological studies

Whole blood for pharmacokinetic analysis was sampled before the administration of study medication, on days 1 and 4, and weekly thereafter. Furthermore, a blood sample was taken at the end of treatment and 4 weeks later. If possible, additional blood samples were collected 1, 2 and 3 weeks after the end of treatment. Whole blood (5 ml) was collected by venipuncture in 10 ml citrate tubes. Samples were placed on ice and centrifuged at 1500*g* for 10 min. The plasma layer was removed immediately and transferred into labelled polypropylene tubes. Samples were frozen and stored at -20 °C until analysis by LC–mass spectrometry (LC–MS).

The plasma sample clean-up procedure was performed by automated solid-phase extraction on 1-ml phenyl cartridges. Miltefosine was used as an internal standard. An aliquot of the eluate solvent was directly injected onto the silica column (Chromspher 5Si, 100×3 mm ID). Subsequently, plasma levels of perifosine were determined by high-performance liquid chromatography in combination with mass spectrometry. The column outlet was connected to the TurbolonSpray sample inlet (Sciex, Thornhill, Canada) without splitting. Ions were created at atmospheric pressure and were transferred to an API 365 triple quadrupole mass spectrometer. The transitions for perifosine and the internal standard miltefosine were selected from m/z 462.4 to 112.0 and 408.2 to 124.8, respectively. The lower limit of quantitation (LLQ) was 4.0 ng/ml using a 250-µl sample volume. The assay was validated up to a concentration of 2000 ng/ml [31]. The terminal half-life of perifosine was determined by mono-exponential regression of the concentrations measured in the weekly plasma samples during the post-treatment follow-up period.

3. Results

3.1. Patients

22 patients were entered in this study, 12 men and 10 women. The median age was 53 years (range 26–70 years). All patients had advanced solid tumours, the majority in the colon (41%), and all but 2 patients had received prior therapy. Further patient characteristics are outlined in Table 1. The administered daily doses ranged from 50 to 350 mg. 8 patients received two courses, while 10 patients went off study after one course, due to either progressive disease (2 patients, treated at dose levels 4 and 6), intolerable side-effects (6 patients, 1 treated at dose level 2, 1 at dose level 4, 3 at dose level 5 and 1 at dose level 6), clinical deterioration (1 patient, treated at dose level 3) or a serious adverse event (1 patient, treated at dose level 4 who developed fever, which was probably not drug-related). 3 patients received more than two courses: three, five and six, respectively. 1 patient, who suffered from an advanced oesophagus carcinoma, died 5 days after the start of treatment because of hypovo-

Table 1
Patient characteristics

	Patients (*n*)
Total	22
Sex	
Male	12
Female	10
Age (years)	
Median (range)	53 (26–70)
Performance Status	
0	8
1	11
2	3
Tumour type	
Colon	9
Rectum	2
Pancreas	1
Ovarium	1
Liver	1
Eye (melanoma)	1
Oesophagus	1
Bladder	1
Adenocarcinoma of unknown primary	5
Previous therapy	
Surgery	14
Chemotherapy	20
Radiation therapy	7
None	2

lemic shock due to massive tumour haemorrhage and another patient developed fever (probably not related to treatment) and went off the study after 2 days. Both patients were replaced. 21 patients were assessable for toxicity during the first course and 11 during the second.

3.2. Toxicity

No bone marrow toxicity was observed in any of the patients treated. Non-haematological toxicity, however, was reported by many patients and consisted mainly of nausea (52%), vomiting (38%), diarrhoea (43%) and fatigue (43%). One patient developed reversible renal insufficiency, manifesting in increased creatinine levels up to 214 μmol/l, and another developed hypercalcaemia. A clear relationship for these last two adverse events to the study medication could not be established. Toxicities are outlined per dose level in Table 2. The gastrointestinal disturbances were often continuous and did not always respond satisfactorily to standard antiemetic treatment (domperidone or metoclopramide). At dose levels 1 and 2, 1 out of 3 patients required peripherally-acting antiemetics, at dose level 3, 2 out of 3 patients received such treatment, and on dose level 4 all patients treated (4 out of 4) needed daily domperidone or metoclopramide. At dose levels 5 and 6, patients even needed 5HT-3 antiemetic treatment (dose level 5: 5 out of 6 patients, for the sixth patient domperidone sufficed; dose level 6, 1 out of 1 patient). The 5HT-3 antagonists decreased the gastrointestinal disturbances, but could not fully abolish them in all patients. The occurrence, as well as the severity, of the gastrointestinal side-effects appeared to increase with increasing doses. In addition, the reported fatigue was often persistent in nature and experienced as very inconvenient by most patients. For these reasons, no further dose escalation was performed and patient inclusion was halted, even though MTD had not been reached according to the employed definition. Based on the rate of patients who dropped out of the study due to side-effects (Table 3), the MTD was established at 200 mg/day. At this dose-level, 1 out of 5 patients went off study because of the reported side-effects, whereas at the next higher dose (250 mg/day) this rate was 3 out of 6 patients. Clearly, further dose escalations could not be implemented because of side-effects resulting in early treatment discontinuation in the majority of patients.

Table 2
Toxicities with their gradings according to the Common Toxicity Criteria (CTC) per dose-level

Daily dose (mg)		50	100	150	200	250	350	Total
Patients (*n*)		3	3	3	5	6	1	21
	CTC grade							
Nausea	1–2	–	1	1	2	5	1	10
	3–4	–	1	–	–	–	–	1
Vomiting	1–2	–	1	–	2	4	1	8
	3–4	–	–	–	–	–	–	–
Anorexia[a]		–	1	–	–	–	–	1
Abdominal pain[a]		–	–	1	1	–	–	2
Diarrhea	1–2	–	–	2	1	4	1	8
	3–4	–	–	–	–	1	–	1
Fatigue[a]		–	1	2	1	4	1	9
Stomatitis[a]		–	–	1	–	–	–	1

[a] Anorexia, abdominal pain, fatigue and stomatitis only occurred at CTC grades 1 and 2.

3.3. Pharmacological studies

Plasma concentrations of perifosine were measured in all patients. However, as mentioned before, 2 patients went off study before completing 1 week of treatment, and these patients were excluded from all calculations. Representative plasma concentration versus time curves of the first cycle on each dose-level are depicted in Fig. 2. Dose level 6 (350 mg/day) is not depicted, because only 1 patient had been included at this level, and sampling of this patient was incomplete. Mean predose con-

Table 3
Patients who discontinued treatment before completing the 2-month target period due to side-effects

Patient (*n*)	Daily dose (mg)	Treatment duration (courses)	Side-effect (CTC grade)
7	100	1	Nausea (3), vomiting (2)
11	200	1	–[a]
17	250	1	Nausea (2), vomiting (1), diarrhoea (3), fatigue (1)
19	350	1	Nausea (2), vomiting (1), diarrhoea (2), fatigue (1)
20	250	1	Nausea (1), vomiting (2), diarrhoea (2), fatigue (2)
22	250	1	Nausea (2), vomiting (1)

[a] This patient went off study for two reasons: intercurrent disease and patient refusal due to lack of tolerability.

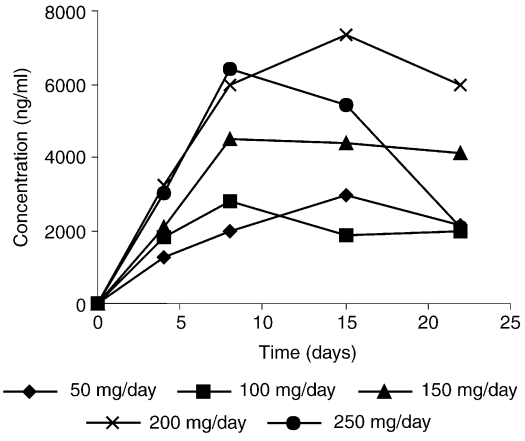

Fig. 2. Trough plasma concentrations of perifosine versus time during cycle 1. Representative curves for each dose level.

centrations of perifosine on days 4, 8, 15 and 22 of the first cycle are outlined per dose level in Table 4. A positive correlation between dose and pharmacokinetic parameters for perifosine was suggested. The plasma concentrations of perifosine on day 4 of cycle 1 versus the administered dose are given in Fig. 3. Regression analysis revealed a linear relationship (correlation coefficient = 0.62, $P = 0.005$). From 1 patient (included at dose level 5), samples were taken from more than two cycles, the results of which are shown in Fig. 4. As can be seen, some accumulation between, as well as within, cycles occurs. From the weekly plasma samples drawn during the posttreatment follow-up period of this patient, a terminal half-life of 105 h was calculated.

4. Discussion

In our phase I trial, nausea and vomiting, as well as fatigue, were the toxicities which precluded further dose escalation. The gastrointestinal disturbances occurred at each dose level, and were the main reason for the high early drop-out of patients. Prophylactic use of antiemetics (domperidone, metoclopramide or even granisetron) as well as taking the medication together with a meal, could not adequately abolish the nausea and vomiting in most patients. From this study, it appears as if perifosine has a similar toxicity profile as the parent compound miltefosine. One patient also suffered from renal dysfunction, which has been reported with oral miltefosine as well [21,22]. In the case of miltefosine, the renal impairment was found to be reversible [21]. Our patient responded well to hydration, which was combined with cessation of treatment, and serum creatinine values returned quickly to normal. A rechallenge with perifosine was not possible, because the affected patient died from a, not drug-related, heart condition shortly afterwards. The MTD of perifosine, recommended for phase II testing, was established at 200 mg/day, based on the drop-out rate of patients who did not tolerate treatment. Because perifosine exerts its antineoplastic action through interference with signal transduction, which is a continuous process, the best results of treatment might be expected from prolonged administration [32]. Hence, for perifosine as well as all other agents acting through such a mechanism, tolerability of treatment is of high importance, probably more so than is the case for classical cytotoxic drugs, which are usually administered intravenously on intermittent dosing schemes. Apart from fatigue, perifosine lacked non-gastrointestinal toxicities. Especially the absence of influence on bone marrow function holds promise for future studies where perifosine, or other ether lipids, are combined with classical chemotherapeutics or radiotherapy [9]. The pharmacological studies revealed a long terminal half-life of perifosine and some accumulation on this dosing scheme. However, due to the small number of patients who could be analysed, further research is warranted. In a panel of human tumour cell lines, the IC_{50} of perifosine ranged from 0.2 to 19.9 μmol/ml, corresponding to 0.09–9.2 mg/ml [28]. The trough levels obtained in our phase I trial on the recommended dose for phase II testing, 200 mg/day, ranged from 2595 to 8195 ng/ml. Hence, the achieved concentrations are most likely within the *in vitro* bioactive range. As yet, little is known about the excretion and metabolism of perifosine. An ongoing phase I trial, which doses perifosine on a weekly schedule, includes urine as well as plasma pharmacokinetic sampling. Preliminary results of this trial have demonstrated that renal excretion of perifosine is most likely minimal [29]. In our trial, hints of activity for perifosine were seen in 2 of 16 patients evaluable for antitumour response. One patient with liver cell carcinoma and 1 patient with an eye melanoma metastasised to the liver, showed stable disease which lasted for 4 months in both cases, as confirmed by computerised tomography (CT)-scans. These patients were treated at dose levels 4 and 6, respectively.

In conclusion, the frequent occurrence of gastrointestinal disturbances hampered chronic treatment with perifosine in our phase I trial. This is considered a major limitation, because chronic daily treatment is currently thought to be optimal for this type of drug that interferes with signal transduction pathways. Other phase I trials are being executed with perifosine administered in different dosing schedules, the results of which are

Table 4
Predose concentrations of perifosine on different days during course 1 per dose level, represented as means and (standard deviations)

Daily dose (mg)	Concentration perifosine (ng/ml)							
	Day 4	*n*	Day 8	*n*	Day 15	*n*	Day 22	*n*
50	774 (467)	4	1392 (841)	3	1797 (1173)	3	1607 (782)	3
100	2359 (998)	3	3887 (1519)	2	3587 (1891)	3	3347 (1191)	3
150	2797 (611)	3	5154 (1842)	3	4683 (931)	3	4909 (1016)	3
200	3172 (460)	4	4580 (2771)	5	5916 (1428)	3	5651 (1862)	4
250	3906 (2457)	5	6734 (3876)	5	4089 (1309)	3	4615 (1116)	2
350	4347	1	5795	1	6374	1	–[a]	–

[a] No data available.

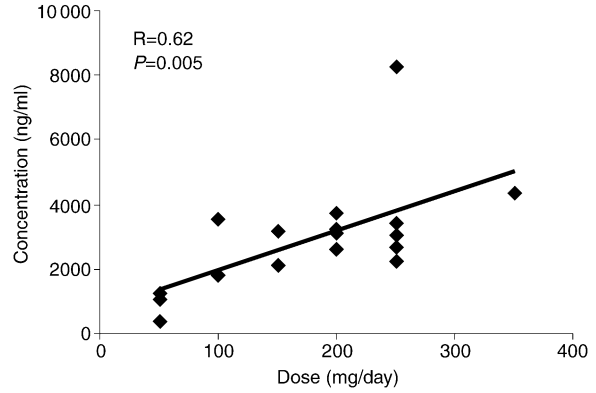

Fig. 3. Trough plasma concentrations of each patient on day 4 of cycle 1 versus administered dose of perifosine.

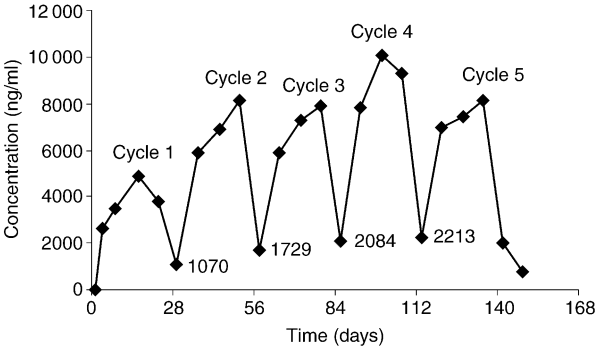

Fig. 4. Weekly measured plasma concentrations of patient 18 (treated at 250 mg/day) over five cycles.

awaited shortly. We have recently started a phase I trial exploring the combination of daily perifosine with radiotherapy. Considering the potential interaction between perifosine and radiotherapy, we employ an escalating dose schedule of perifosine, starting at 50 mg/day.

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