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Increases in Leucocyte and Platelet Counts Induced by the Alkyl Phospholipid Hexadecylphosphocholine

L.C. Pronk, A.S.Th. Planting, R. Oosterom, T.E. Drogendijk, G. Stoter and J. Verweij

Hexadecylphosphocholine (HePC) is a new alkyl phospholipid that has been shown to have antitumour activity *in vitro* and *in vivo*. *In vivo* studies have shown absence of bone marrow toxicity at therapeutic doses. In addition, at the highest dosage group in rats, an increase in white blood cell counts (WBC) was observed. To study the presence of a similar phenomenon in man, frequent measurements of haematological parameters were performed in a series of phase II studies. 70 patients were treated with daily doses of 100–200 mg of the oral formulation of HePC. WBC and platelet counts were performed weekly. In a subgroup of 23 patients serum levels of haemopoietic growth factors were measured before and during treatment. A significant increase in WBC and platelet counts was seen in 74 and 73% of the patients, respectively. In 4 patients, bone marrow showed normal cellularity, and in 1 patient, bone marrow culture showed normal numbers and sizes of colony forming units. No abnormal levels or trends over time of cytokines were observed. We conclude that oral HePC induces an increase in WBC and platelet counts in the majority of those treated.

Key words: cytokines, hexadecylphosphocholine, leukocytosis, thrombocytosis
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INTRODUCTION

ETHER LIPIDS and related drugs have recently entered the stage of clinical testing. Hexadecylphosphocholine (HePC) is a new alkyl phospholipid with antitumour activity in human leukaemic cell lines, and in methylnitrosurea (MNU)-induced and dimethylbenzanthracene (DMBA)-induced breast carcinoma models in the rat [1–6].

In vivo studies in mice and rats showed absence of bone marrow toxicity at therapeutic doses [1, 2, 5, 6]. In addition, at the highest dosage in rats, a significant increase in the total white blood cell (WBC) counts was noted, mainly related to an increase in the absolute number of granulocytes [1, 2, 4, 5]. Bone marrow examinations did not show augmentation of granulocyte precursor cells [2]. To study the presence of a similar effect in man, we prospectively analysed WBC and platelet counts in 70 patients included in phase II studies, in which the oral formulation of HePC was administered. In a subgroup of patients, serum haemopoietic growth factor levels were measured before and during treatment with HePC.

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PATIENTS AND METHODS

Patients

Phase II studies were performed with oral HePC in non-small cell lung cancer, colorectal cancer, squamous cell head and neck cancer and soft tissue sarcomas.

Criteria for inclusion were histological proof of malignancy, performance status WHO $\leq$ 2, oral informed consent, age $\geq$ 18

years, leucocyte count > 3.0 × 10^9/l, platelet count > 100 × 10^9/l, serum creatinine < 120 μmol/l or creatinine clearance > 60 ml/min and serum bilirubin < 30 μmol/l.

In patients with non-small cell lung cancer, no prior chemotherapy was allowed. In patients with colorectal cancer, head and neck cancer, and soft tissue sarcoma only one prior chemotherapy regimen was permitted.

HePC (ASTA Medica AG, Frankfurt, Germany) was provided as gelatine capsules containing 50 mg of the drug. For patients with colorectal, non-small cell lung and head and neck cancer, a dose escalation was included in the studies. Patients started taking the capsules twice daily after meals which, in absence of nausea and/or vomiting, after 1 week was escalated to three times a day and if still feasible to four times daily after another week. In the soft tissue sarcoma study, a fixed dose of three times daily was used.

WBC and platelet counts were performed weekly. For the purpose of this analysis, patients were considered evaluable if they had received at least 2 weeks of treatment. Cut-off point for the present analysis was a maximum treatment duration of 8 weeks.

Table 1. Patients' characteristics

	No. of patients
Total number of patients	72
Male/female	45/27
Age (years)	
Median	57
Range	25–79
Performance score (WHO)	
Median	1
Range	0–2
Primary tumour	
Colorectal	33
Non-small cell lung	16
Head and neck	12
Soft tissue sarcoma	11
Pretreatment	
Chemotherapy	18
Immunotherapy	2
None	52

Methods

WBC and platelet counts were determined according to the flow cytometric principle (Haematology analyses H*1, Bayer-Technicon, Gorinchem, The Netherlands). Leucocyte counts were carried out with the H*1; in the case of abnormal results, a microscopic differential count of 100 leucocytes was performed. Actual values were recorded, as well as the percentages related to the pretreatment values. Serum levels of the haematopoietic growth factors granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), interleukin (IL)-1β, IL-3, and interferon-γ (IFN-γ) were measured before treatment, and after 2 and 4 weeks of treatment just prior to the first daily dose. For the measurement of human G-CSF and IL-3, the Quantikine sandwich enzyme immunoassays from Research and Diagnostic Systems (Minneapolis, U.S.A.) were used. Human GM-CSF, IL-1β, and IFN-γ were measured with immunoradiometric assays obtained from Medgenix Diagnostics (Fleurus, Belgium).

The normal serum values of these cytokines are: G-CSF < 120 ng/l; GM-CSF <15 ng/l; IL-1β < 15 ng/l; IL-3 < 30 ng/l; IFN-γ < 0.5 kU/l (normally not detectable in serum).

Statistical analysis

A paired *t*-test and a Wilcoxon matched pairs signed ranks test were performed to test for a difference between the WBC counts of each week and the WBC counts of week zero. The same procedure was followed for the platelet counts.

RESULTS

72 patients were treated with the oral formulation of HePC. The patients' characteristics are shown in Table 1. 2 patients received the drug for less than 2 weeks. 70 patients were treated for 2 or more weeks, and were considered evaluable for the effect of the drug on WBC and platelets. The median duration of treatment was 6 weeks, the median daily dose was 150 mg (range 100–200).

In 52 patients (74%), a consistent rise in WBC was seen, ranging from a 1.2-fold to a 3.9-fold increase (median 1.52); the median highest WBC was 11.5 × 10^9/l (range 5.1–24.4). In 51 patients (73%), a consistent increase in platelet counts was found, ranging from a 1.19-fold to a 2.33-fold increase (median 1.51); the median highest platelet count was 475 × 10^9/l (range 181–1176).

In 43 patients (61%), an increase in WBC and platelet counts was observed. In 9 patients (13%), an increase in WBC was seen without an increase in platelet counts. 8 patients (11%) showed an increase in platelet counts without an increase in WBC. In 10 patients (14%), there was no increase in WBC or platelet counts.

Figure 1 shows that, in comparison with baseline levels, the mean WBC increased until week 5 and then remained more or less stable afterwards. These increases were statistically significant.

Differential counts were not asked for by protocol and were thus not consistently performed. However, in those cases where differential counts were available, the increase in WBC appeared mainly to be related to an increase in granulocytes.

In comparison to baseline levels, the mean of the platelet counts increased until week 4 and then stabilised, as seen in

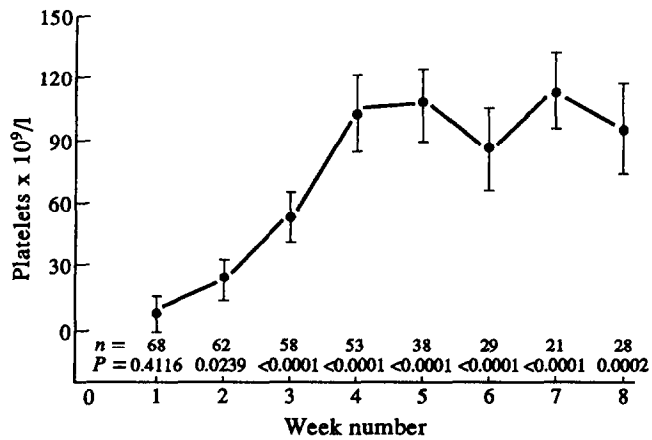

Figure 1. The change in WBC in comparison to baseline levels is plotted, together with standard error bars. *n* is the number of observations and *P* is the *P* value calculated with a two-sided paired *t*-test.

Figure 2. These increases were statistically significant, except for week 1 compared with week 0.

In 4 patients with elevation of the WBC platelet counts, a bone marrow biopsy was performed, which did not reveal any abnormal cellularity. In 1 patient, a bone marrow culture was performed which showed normal colony forming units (CFU) by size and number.

In 23 consecutively entered patients, serum levels of G-CSF, GM-CSF, IL-1β, IL-3 and IFN-γ were measured before the start of treatment and after 2 and 4 weeks of treatment. HePC has a half life of 2–3 days [1]. Steady state serum levels are achieved after approximately 1 week. Hypothesising that the drug might have an influence on cytokine production, serum levels were measured before the first daily dose and after 2 and 4 weeks. In 12 of these patients (52%), a leuco- and thrombocytosis was seen. In 3 patients (13%) only leucocytosis was observed, while in 2 patients (9%) only thrombocytosis was seen. In 6 patients (26%), there was no rise in WBC or platelet counts.

In most of the patients, the serum levels of cytokines were undetectable; in some, the levels were high prior to the start of therapy. However, a trend over time of serum cytokine levels could not be detected (data not shown).

DISCUSSION

HePC is a new alkyl phospholipid that has shown antitumour activity *in vitro* and *in vivo* [1–6]. In contrast to many cytotoxic drugs, HePC does not interfere with the cell nucleus but is thought to interfere with the cell membrane [1, 2, 6, 7].

In vivo studies have shown absence of bone marrow toxicity at therapeutic doses [1, 2, 5, 6]. In addition, an increase in the total WBC count has been seen at the highest dosage group in DMBA-induced breast carcinoma in the rat, mainly related to an increase in the absolute number of granulocytes [1, 2, 4, 5], although bone marrow examination did not show an increase in granulocyte precursor cells [2]. Therefore, it was suggested that recruitment of the peripheral white blood cell pool may be responsible for the observed phenomenon [1, 2, 4, 5]. Similar changes in platelet counts were not observed in these studies. We performed clinical studies with daily oral dosing of HePC [8–10]. The effect of the drug on the WBC and platelets was evaluated in 70 patients. In 74% of the patients, a significant increase in WBC counts was seen, while in 73% a rise in platelet counts was found. To determine if this was related to an increase in the progenitor cells, bone marrow aspirations were performed in 4 patients with leuco- and/or thrombocytosis, but these did not reveal abnormal cellularity. In 1 patient, a bone marrow culture showed normal CFUs by number and size. Serum levels of cytokines were measured before and during treatment with HePC, in a subgroup of patients, to examine whether the leuco- and/or thrombocytosis could be explained by an induced rise in serum haematopoietic growth factor levels. No abnormal serum levels or trends over time of these growth factors were found. However, this only excludes major changes in these levels in peripheral blood. No information was obtained on local production of these factors.

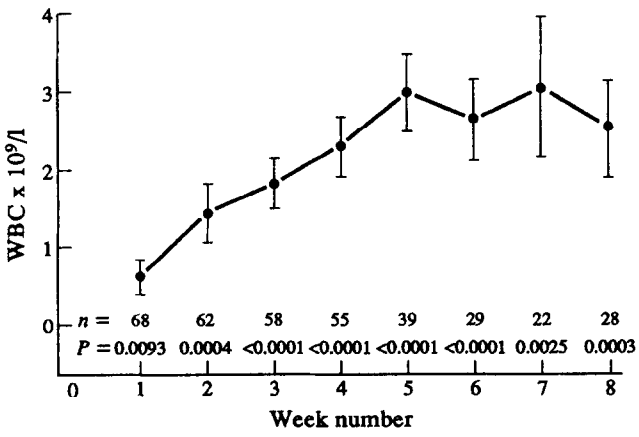

Figure 2. The change in platelet counts in comparison to baseline levels is plotted, together with standard error bars. *n* is the number of observations and *P* is the *P* value calculated with a two-sided paired *t*-test.

Thus, although HePC induces an increase in WBC and platelet counts in the majority of patients treated, the additional investigations could not identify the underlying mechanism. In this study, we did not examine whether prolonged survival time or peripheral recruitment of leucocytes and platelets accounts for the increase in WBC and platelet counts. In phase I studies, a moderate increase in leucocytes was seen during treatment with HePC [11, 12]. Vehmeyer and colleagues investigated whether the leucocytosis could be explained by a HePC-enhanced CSF-dependent stimulation of bone marrow progenitor cells [12]. Bone marrow from 3 patients with chronic myeloid leukaemia (CML) and 6 patients without known haematological disorders was examined. Colony formation was investigated in the presence of haematopoietic growth factors, G-CSF, GM-CSF and IL-3, either as single agents or in combination. Incubations were performed by omission of HePC and in the presence of increasing concentrations of HePC. Without G-CSF, GM-CSF and IL-3, HePC did not induce colony formation. It could be concluded that HePC has no mitogenic activity by itself. HePC with G-CSF showed the most impressive effect, with a stimulating activity of up to eight times greater than in controls. This effect was seen in patients with CML as well as in patients without a haematological disorder. Higher concentrations of HePC suppressed the growth of G-CSF- and GM-CSF-induced colony formation, while at low doses, HePC appeared to act as a co-stimulator. Because preliminary data from clinical pharmacokinetics of HePC show that, with the used oral dosing regimen, only low plasma levels can be achieved (ASTA Medica AG, data on file), this may be related to the clinically observed increases in WBC and/or platelet counts.

Nooter and colleagues also concluded, from *in vitro* studies on mouse bone marrow cells, that HePC has a growth enhancing effect on clonogenic haemopoietic progenitor cells, including the pluripotent stem cell, by direct action in synergy with specific growth factors [13]. In their study, the growth enhancing phenomenon was most pronounced with IL-3, or GM-CSF, with a similar bell-shaped dose-dependent curve as reported by Vehmeyer [12].

Although the precise mechanism of HePC-stimulated growth factor-dependent colony formation still has to be determined, we conclude that oral HePC induces an increase in WBC and platelet counts in the majority of patients treated.

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In vitro Activity of 2-Chlorodeoxyadenosine (CdA) in Primary Cultures of Human Haematological and Solid Tumours

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2-Chlorodeoxyadenosine (CdA) is a deaminase-resistant purine analogue which has shown clinical activity against various haematological tumours, and is currently undergoing phase II trials. In the present study, the semiautomated fluorometric microculture cytotoxicity assay (FMCA) was used for *in vitro* evaluation of CdA activity in cell suspensions from both haematological and solid tumours. A total of 133 samples from various diagnoses were successfully tested with continuous drug exposure. CdA showed high *in vitro* activity against samples from chronic and acute lymphocytic leukaemia and acute myelocytic leukaemia, but little or no response was observed in the solid tumour groups. Cross-resistance analysis with standard drugs revealed the following rank order of correlation coefficients: cytosine arabinoside (AraC) > daunorubicin > doxorubicin > vincristine > prednisolone > 4-hydroperoxycyclophosphamide > etoposide > cisplatin. The high correlation between CdA and AraC was maintained even if the analysis was based only on the haematological tumours. The results indicate that CdA is differentially active against haematological tumours with little or no activity against solid tumours. CdA also appears highly cross resistant with AraC. If this disease-specific information is substantiated in further clinical trials and extended to other phase I–II drugs, non-clonogenic drug resistance assays such as the FMCA may become useful in new drug evaluation, and in targeting specific diagnoses and patients for phase II trials.

Key words: 2-chlorodeoxyadenosine, drug resistance, drug screening
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INTRODUCTION

2-CHLORODEOXYADENOSINE (CdA) IS a novel adenosine deaminase-resistant purine analogue of deoxyadenosine [1, 2]. It has recently emerged as the drug of choice for the treatment of hairy cell leukaemia [3–5], and has promising activity in chronic lymphocytic leukaemia [6, 7] and low grade non-Hodgkin's lymphoma [8]. CdA is taken up by cells and converted by deoxycytidine kinase (dCK) to monophosphate. The triphosphate accumulates in cells with high dCK levels and low dephosphorylating activity of 5'-nucleotidase (5NT) [2]. The mechanism by which the phosphorylated congeners of CdA exert their cytotoxic effects is not fully understood, although apoptosis and/or alteration of DNA repair, secondary to pertuberation of the deoxynucleotide pool with subsequent NAD depletion, have been discussed [2, 9, 10].

Because of the high levels of dCK and low levels of 5NT observed in lymphocytes, CdA was originally conceived to be relatively lymphocyte-specific [1, 2]. However, recent studies have shown that CdA also inhibits growth of myeloid progenitors [11], and the drug also appears active in acute myelocytic leukaemia [12]. The major toxicity encountered in the clinical situation is in fact myelosuppression [8, 12]. This may indicate