

## CLINICAL RESEARCH

## Alfacalcidol as a modulator of growth of low grade non-Hodgkin's lymphomas

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### Abstract

Ten patients with low grade non-Hodgkin's lymphoma (seven follicular small cleaved and three small lymphocytic) were treated with 1 µg oral alfacalcidol (1α-hydroxycholecalciferol) daily. Of the seven patients with lymphomas of follicular small cleaved subtype, one achieved complete and three partial remission, whereas none of three patients with small lymphocytic lymphomas responded. In seven of the 10 patients, 1,25(OH)<sub>2</sub>D<sub>3</sub> receptors were measured in tissue from lymph nodes, and a positive correlation between the presence and amount of receptor and response to alfacalcidol was found.

These preliminary data suggest that alfacalcidol has appreciable antitumour activity in low grade non-Hodgkin's lymphomas.

### Introduction

Low grade non-Hodgkin's lymphomas as defined in the working formulation of non-Hodgkin's lymphomas<sup>1</sup> consist of the following histological subtypes: small lymphocytic, follicular small cleaved cell, and follicular mixed small cleaved cell and large cell. They account for 30-45% of all lymphomas and are generally very responsive to single agent or combination chemotherapy.<sup>2</sup> With the possible exception of the follicular mixed small cleaved cell and

large cell subtype,<sup>3</sup> however, the remission of disease obtained with such chemotherapy is not durable, and a pattern of continual relapse has been identified.<sup>4</sup> Thus although these tumours are compatible with long survival they can rarely be cured, and delaying the introduction of chemotherapy until it is clinically indicated is unlikely to compromise survival.<sup>5</sup> During this treatment free period it is, therefore, reasonable to investigate therapeutic alternatives to chemotherapy.

The interrelation between vitamin D and the lymphomedullary system was recently reviewed<sup>6</sup> after developments in research in this topic over the past five years. During this time 1,25-dihydroxy-vitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) at near physiological doses has been shown to induce in vitro differentiation of murine and human leukaemia along monocyte and macrophage pathways.<sup>7,8</sup> Moreover, this type of differentiation in a human leukaemia cell line is associated with the regulation of an oncogene's expression.<sup>9</sup> An in vivo antitumour effect of alfacalcidol (1α-hydroxycholecalciferol; 1α-OHD<sub>3</sub>) has been shown by the suppression of the growth of sarcoma and Lewis lung tumours in mice,<sup>9a</sup> and mice inoculated with leukaemia cells survive longer when given 1,25(OH)<sub>2</sub>D<sub>3</sub>,<sup>9b</sup> which also suggests in vivo antitumour activity. When this is coupled with the fact that malignant and activated T and B lymphocytes have 1,25(OH)<sub>2</sub>D<sub>3</sub> receptors, which are not found on resting lymphocytes,<sup>10</sup> then the potential of 1,25(OH)<sub>2</sub>D<sub>3</sub> as an antitumour agent in human lymphoma and leukaemia becomes obvious.

The clinical course of low grade non-Hodgkin's lymphoma makes it the ideal type of tumour for investigating such a hypothesis, and the lack of toxicity of alfacalcidol makes a clinical study feasible. We report on the antitumour effect of alfacalcidol in 10 patients with low grade non-Hodgkin's lymphomas.

### Patients and methods

Twelve patients (eight women, four men) with low grade non-Hodgkin's lymphomas entered the study. All had had stable disease for the preceding three months (except one who had had a recent recurrence). When possible each patient underwent lymph node biopsy on entry to the study; half of the specimen obtained was sent for routine histological examination and the remainder for measurement of 1,25(OH)<sub>2</sub>D<sub>3</sub> receptor concentrations. The tissue reserved for measurement of receptor concentrations was immediately frozen in liquid nitrogen and stored at -70°C. Tissue was allowed to thaw on

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ice and a 10% wt/vol homogenate prepared using the buffer and homogenising conditions described by Rheinhardt *et al.*<sup>11</sup> After centrifugation at 100 000 *g* for 60 minutes the supernatant was incubated with increasing concentrations of tritiated and cold 1,25(OH)<sub>2</sub>D<sub>3</sub> for 15 hours at 4°C. Bound and free steroid was separated using dextran coated charcoal,<sup>12</sup> and Scatchard curves were constructed after correction for non-specific binding.<sup>13</sup> Protein concentration was measured by the method of Lowry *et al.*<sup>14</sup>

We measured serum concentrations of urea, electrolytes, calcium phosphate, magnesium, 1,25(OH)<sub>2</sub>D<sub>3</sub>, and parathyroid hormone; serum activities of alkaline phosphatase, aspartate transaminase, and alanine transaminase; full blood count; and erythrocyte sedimentation rate before starting treatment with alfacalcidol 1 µg daily (One-Alpha, Leo Laboratories). These measurements were repeated each time the patient was reviewed (every three to four weeks). At each review response was assessed using the standard criteria of the World Health Organisation.<sup>15</sup> These define complete remission as the absence of all clinically detectable malignant disease for at least four weeks; partial remission as a decrease of more than 50% in tumour size in at least 50% of the affected sites for at least four weeks; stable disease as no change in measurable disease for at least eight weeks; and progressive disease as an increase of more than 50% in measurable disease or the development of any new lesions. The clinician making the assessment did not know the receptor state of the patient. Treatment with alfacalcidol was continued until there was evidence of progression of the disease or toxicity.

## Results

Routine histological examination showed that two patients had intermediate grade lymphomas<sup>1</sup> and were thus unsuitable for treatment with alfacalcidol, but they were included in the table as information about their receptor state was available. The table shows the results of treatment with alfacalcidol. All patients except one (case 1) had normal biochemical values, including 1,25(OH)<sub>2</sub>D<sub>3</sub> and parathyroid hormone concentrations, before and during treatment. In case 1 the patient had mild hypercalcaemia (2.7 mmol/l; 10.8 mg/100 ml) for eight weeks after treatment with alfacalcidol was begun, which spontaneously returned to normal values. The treatment had no other toxic effects. Two patients (cases 9 and 10) had raised white cell counts before treatment (61 × 10<sup>9</sup>/l and 253 × 10<sup>9</sup>/l) due to a mature lymphocytosis consistent with an exfoliating small lymphocytic lymphoma. As the lymphadenopathy in these patients increased during treatment with 1α-OHD, the white cell count rose further.

## Discussion

In this study we showed that alfacalcidol had considerable antitumour activity in the treatment of low grade non-Hodgkin's lymphoma. This effect was restricted to the patients with lym-

phoma of follicular small cleaved cell subtype, but this may reflect the small number of patients with the small lymphocytic subtype studied. Some correlation seemed to exist, however, between the presence and amount of 1,25(OH)<sub>2</sub>D<sub>3</sub> receptor and subsequent response to alfacalcidol. This is important, as this receptor state may ultimately be used to determine a patient's suitability for treatment with alfacalcidol. The duration of treatment required to induce regression of the tumour ranged from three to 16 weeks. Therefore, in the absence of progressive disease or toxicity, we recommend that treatment should be continued for a minimum of six months. Increasing the dose of alfacalcidol to more than 1 µg daily could add to toxicity, but we believe that it may be worth trying this in patients with low receptor concentrations who do not respond to 1 µg daily and those who respond but then relapse.

The precise mechanism by which alfacalcidol produced regression of the tumours in our patients is not clear. Regression may have been mediated through a differentiation effect, analogous to that observed with the human leukaemia cell line, in which leukaemia cells were transformed into monocytes by culture with 1,25(OH)<sub>2</sub>D<sub>3</sub>.<sup>8</sup> It has also been shown, however, that 1,25(OH)<sub>2</sub>D<sub>3</sub> inhibits the production of interleukin 2, a T lymphocyte growth factor, by activated human T lymphocytes<sup>16</sup> and that it can suppress the proliferation of and production of immunoglobulin by normal peripheral mononuclear cells.<sup>17</sup> Thus it is possible, though speculative, that for lymphomas with 1,25(OH)<sub>2</sub>D<sub>3</sub> receptors 1,25(OH)<sub>2</sub>D<sub>3</sub> is an inhibitory growth regulator, mediated by suppression of production of interleukin 2.

Spontaneous regression of tumours is well documented in low grade non-Hodgkin's lymphoma,<sup>5</sup> and it could be argued that the responses to alfacalcidol in this study were simply manifestations of this phenomenon. Spontaneous remission of the magnitude that occurred in our patients would, however, be extremely unusual; a previous review reported an incidence of 5%, generally in untreated patients.<sup>18</sup> Horning and Rosenberg described spontaneous remission in 23% of 83 patients with low grade lymphomas, and in a group with the follicular small cleaved cell subtype the figure was as high as 30%,<sup>5</sup> but the median time to regression was long (eight months) and the patients were highly selected: none had had chemotherapy, and they were included in the study only because at presentation they did not require treatment with chemotherapy.

In our patients there was some evidence that the regression of tumours was due to alfacalcidol, such as the presence of receptors in those who responded and the absence or low level of receptors in those who did not and a fairly good temporal relation between the introduction of alfacalcidol and response. It is interesting to speculate that the spontaneous regression described by Horning and

### Response to alfacalcidol and 1,25(OH)<sub>2</sub>D<sub>3</sub> receptor concentrations

Case No	Sex, age	Histology, date of diagnosis	Previous treatment	Measurable disease	Response	Duration of response (time taken to respond)	Receptor level (fmol/mg)
1	F, 55	FSC stage IVA, April 1982	None	Lymphadenopathy, splenomegaly	Complete remission	30 weeks (4 weeks)	Not done
2	F, 44	FSC stage IIIA, July 1978	CHOP, radiotherapy	Right axillary lymphadenopathy, para-aortic nodes on computed tomography	Partial remission	38 weeks (16 weeks)	2.02
3	F, 45	FSC stage IVA, December 1973	MVPP, CVP	Splenomegaly, para-aortic nodes on computed tomography	Partial remission	29 weeks (3 weeks)	1.32
4	M, 61	FSC stage IIIA, December 1983	Radiotherapy	Lymphadenopathy	Partial remission	16 weeks (8 weeks)	4.07
5	F, 54	FSC stage IVB, October 1971	MVPP, chlorambucil	Lymphadenopathy, hepatosplenomegaly	Progressive disease		Not detected
6	M, 59	FSC stage IVB, May 1981	MVPP, chlorambucil	Lymphadenopathy, hepatosplenomegaly	Progressive disease		Not detected
7	F, 47	FSC stage IVA, January 1984	None	Pulmonary deposits	Progressive disease		No tissue available
8	F, 55	SL stage IVA, June 1984	None	Lymphadenopathy, splenomegaly	Stable disease		0.8
9	M, 51	SL stage IVA, June 1976	Chlorambucil	Lymphadenopathy, hepatosplenomegaly	Progressive disease		Not done
10	M, 50	SL stage IVB, November 1978	MVPP, chlorambucil	Lymphadenopathy, hepatosplenomegaly	Progressive disease		0.37
11	F, 63	FSC stage IIIA, January 1983	Ngne	Lymphadenopathy	Not tested because transformation to intermediate grade lymphoma occurred		1.53
12	F, 45	FSC stage IIIA, August 1977	MVPP	Lymphadenopathy	Not tested because transformation to intermediate grade lymphoma occurred		0.28

FSC=Follicular small cleaved cell subtype. SL=Small lymphocytic subtype. CHOP=Cyclophosphamide, doxorubicin, vincristine, prednisone. MVPP=Mustine, vinblastine, procarbazine, prednisone. CVP=Cyclophosphamide, vincristine, prednisone.

Rosenberg in patients with lymphomas in the United States may have been partly due to an effect of vitamin D, as the normal range for vitamin D is known to be higher in the United States than Great Britain and ingestion of multivitamin preparations is fairly common in the general population in the United States<sup>19</sup> and may be more common among patients with cancer.

We cannot make any useful comment on the effect that alfacalcidol might have on the survival of patients from this study. Clearly this would require longer follow up and more patients in a randomised study. Nevertheless, if alfacalcidol could be substituted for cytotoxic treatment as a means of controlling disease without necessarily affecting survival this would represent a major advance in the treatment of low grade lymphomas: the replacement of a toxic treatment with a fairly non-toxic treatment is obviously beneficial, and more effective treatment of transformed low grade lymphomas might be achieved, as it has been suggested that these tumours respond so poorly to chemotherapy partly because they acquire resistant cell populations during exposure to chemotherapy in the low grade state.<sup>20</sup> Furthermore, the substitution of chemotherapy with alfacalcidol might reduce the risk of secondary leukaemia in patients with lymphoma because this risk is related to the duration of treatment with alkylating agents.<sup>21</sup>

These preliminary data suggest that the antitumour effect of alfacalcidol is not limited to in vitro systems or animal tumour models and that the drug may have an important role as a biological modifier in the management of non-Hodgkin's lymphomas.

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# Treatment with danazol and plasma glucagon concentration

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## Abstract

The association between treatment with danazol and hyperglucagonaemia was studied. Plasma glucagon concentrations were measured during an oral glucose tolerance test in seven women taking danazol and six healthy controls not taking danazol. Results showed that treatment with danazol is associated with severe hyperglucagonaemia, and in three patients glucagon concentrations reached the range suggestive of glucagonoma.

It is important to recognise that this increasingly used drug may cause severe hyperglucagonaemia to prevent patients treated with danazol undergoing unnecessary investigations to localise glucagonoma.

## Introduction

Grossly increased plasma glucagon concentrations (>50 pmol/l (>17.4 ng/ml)) were found fortuitously in a woman receiving danazol for premenstrual exacerbations of lupus erythematosus. Known causes of hyperglucagonaemia were excluded. Glucagon concentrations were found to parallel the dosage of danazol, suggesting that hyperglucagonaemia was due to the drug. To explore this possibility we measured plasma glucagon concentrations during an oral glucose tolerance test in seven women taking danazol and six healthy controls.

## Patients, methods, and results

Seven women aged 20-45 (mean 33.9) years treated with danazol (100-600 mg/day for four to 24 weeks) for endometriosis (five patients) or mastalgia (two patients) were randomly selected from the gynaecological outpatient clinic. One patient was taking phenobarbitone and lorazepam for epilepsy and another norethisterone 20 mg/day. Body weight ranged from 45.5 to 82.7 kg (mean 62.7 kg). The six control women did not differ noticeably from the group treated with danazol in either age (range 23-40, mean 29.8 years) or body weight (range 50 to 68 kg, mean 58.3 kg); one was taking an oral contraceptive.

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