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A Phase I clinical study of the antipurine antifolate lometrexol (DDATHF) given with oral folic acid

Sudsawat Laohavinij,* Stephen R. Wedge*, Micheal J. Lind, Nigel Bailey, Alison Humphreys, Madeleine Proctor, Fiona Chapman, Dorothy Simmons, Avril Oakley, Lesley Robson, Lyndsey Gumbrell, Gordon A. Taylor, Huw D. Thomas, Alan V. Boddy, David R. Newell and A. Hilary Calvert

Cancer Research Unit, The Medical School, University of Newcastle-upon-Tyne, Framlington Place, Newcastle-upon-Tyne, NE2 4HH, UK

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Summary

Lometrexol is an antifolate which inhibits glycinamide ribonucleotide formyltransferase (GARFT), an enzyme essential for *de novo* purine synthesis. Extensive experimental and limited clinical data have shown that lometrexol has activity against tumours which are refractory to other drugs, notably methotrexate. However, the initial clinical development of lometrexol was curtailed because of severe and cumulative antiproliferative toxicities.

Preclinical murine studies demonstrated that the toxicity of lometrexol can be prevented by low dose folic acid administration, i.e. for 7 days prior to and 7 days following a single bolus dose. This observation prompted a Phase I clinical study of lometrexol given with folic acid supplementation which has confirmed that the toxicity of lometrexol can be markedly reduced by folic acid supplementation. Thrombocytopenia and mucositis were the major toxicities. There was no clear relationship between clinical toxicity and the extent of plasma folate elevation.

Associated studies demonstrated that lometrexol plasma pharmacokinetics were not altered by folic acid administration indicating that supplementation is unlikely to reduce toxicity by enhancing lometrexol plasma clearance.

The work described in this report has identified for the first time a clinically acceptable schedule for the administration of a GARFT inhibitor. This information will facilitate the future evaluation of this class of compounds in cancer therapy.

Introduction

Lometrexol (5,10-dideaza-5,6,7,8-tetrahydrofolate-(6R)-DDATHF) is a new folate analogue which was synthesized in 1985 by Taylor and colleagues [1]. Unlike methotrexate, lometrexol does not inhibit dihydrofolate reductase, but acts instead against glycinamide ribonucleotide formyltransferase (GARFT), an enzyme essential for *de novo* purine synthesis [2]. Both *in vitro* and *in vivo*, lometrexol has been shown to have antitumor activity against murine and human tumour cells [2–4], and on the basis of its preclinical activity was selected for clinical trial.

In previous Phase I studies of lometrexol when given alone, the total dose of lometrexol which could be safely given was found to be only 10–12 mg/m^2 *per* course [5–7]. In marked contrast, in mice, 600 mg/m^2/week was tolerated in chronic toxicity studies [8]. Furthermore, the onset of profound myelosuppression and/or mucositis in most patients 6–8 weeks after lometrexol administration prevented administration of more than two courses of therapy in most studies. Thus, it has not been possible to perform Phase II studies to evaluate the potential efficacy of lometrexol. However, evidence of antitumor activity was observed in the Phase I clinical studies of lometrexol, in patients with

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malignant fibrous histiocytoma [9], non-small cell lung cancer, breast cancer and colorectal carcinoma [6].

Following the initial clinical evaluation of lometrexol, further studies were performed in mice in an attempt to ameliorate the cumulative toxicity of lometrexol and hence enable repeated courses of the drug to be given. These studies revealed that the therapeutic efficacy and toxicity of lometrexol were highly dependent upon dietary folic acid intake [10, 11] and these preclinical data prompted the Phase I study of lometrexol given with folic acid supplementation described here. The objectives of this clinical Phase I study were:

(a) To evaluate the effect of folic acid on lometrexol pharmacodynamics, in order to determine whether folic acid improves tolerance of lometrexol.
(b) To determine the toxicity of lometrexol in patients receiving multiple courses of the drug with folic acid supplementation.
(c) To describe the pharmacokinetics of lometrexol in patients receiving folic acid supplementation.

Patients and methods

Patient eligibility

From September 1991 to December 1995, 43 patients with a histologically confirmed diagnosis of malignant solid tumour, which was refractory to established therapies or for which no standard therapy existed, were entered into this study. All patients had a predicted life expectancy of at least 12 weeks, and had recovered from the toxicity of previous treatment before entering onto the study. Specifically, patients were required to not have received previous anticancer therapy or other investigational drugs within at least 4 weeks (6 weeks if prior therapy included a nitrosourea, mitomycin C or extensive radiotherapy). Exclusion criteria included factors which could have interfered with lometrexol disposition/toxicity or folic acid absorption, and comprised; (a) concomitant medication with allopurinol, probenecid, nephrotoxic agents, trimethoprim, anti-epileptics, co-trimoxazole or pyrimethamine, (b) extensive radiotherapy and (c) inflammatory ulcerative bowel disease, or malabsorption syndrome. Concurrent treatment with other experimental drugs or other anticancer therapies was not allowed. Patients with clinical evidence or symptoms suggestive of coronary artery disease or central nervous system disease were excluded. Patients with effusions and/or ascites were also not recruited.

All patients were required to have adequate organ function prior to treatment, with marrow function characterised by a white blood cell count of at least 4×10^9/l, neutrophil count at least 2×10^9/l, haemoglobin level of at least 10 g/dl, and platelet count of at least 100×10^9/l. Adequate hepatic function was also required, as characterised by bilirubin levels of < 25 μmol/l, alkaline phosphatase ≤ 2.5 times upper limit of normal, alanine transaminase (ALT/SGPT) $\leq$ twice the upper limit of normal, prothrombin and partial thromboplastin time within normal range. The creatinine level was required to be less than 120 μmol/l and the glomerular filtration rate (GFR) to be above 50 ml/min as measured by ^{51}Cr-EDTA clearance.

Study design

Folic acid (Approved Prescription Services Ltd., Leeds, U.K.) was given daily as a single 5 mg tablet for 7 days prior to and 7 days following lometrexol administration at 4 week intervals. Lometrexol (Lilly Research Centre, Erl Wood Manor, Surrey, U.K.) was reconstituted in 0.9% (w/v) saline and administered as a rapid i.v. bolus over 30 seconds to one minute at a concentration of 1–10 mg/ml. Patients were admitted to the Department of Medical Oncology, Newcastle General Hospital, to receive lometrexol and were observed for a further 24 hours following drug administration, to ensure that acute toxicity was not apparent. The following studies were performed weekly: physical examination, toxicity and performance status assessment, and biochemical analysis. Full blood counts were measured twice a week. As part of the Phase I trial of lometrexol with folic acid it was important to demonstrate that plasma folate concentrations of patients were increased by folate supplementation and folate levels were measured on course 1 prior to supplementation (day 7) and after 7 days of folate administration but prior to lometrexol (day 0). Plasma folate concentrations were determined using a commercial folate binding assay (SimulTRAC-SNB, Becton Dickinson, Oxford, UK).

The starting dose was 12 mg/m^2 as this dose of lometrexol given alone had been well tolerated on the first course of therapy in previous Phase I studies, regardless of schedule [5–8]. Lometrexol was given as a single bolus injection every 4 weeks, with 5 mg/day oral folic acid administration 7 days prior to treatment with lometrexol and 7 days afterwards on each course. Toxicities were evaluated according to World Health Organisation (WHO) criteria. If repeated courses at a given dose level were tolerated without toxicity greater

than WHO grade II, doses were escalated according to the clinical judgement of the investigator with the approval of the Medicines Control Agency (London, UK). Doses were initially escalated to 16, 30 and 45 mg/m^2 every 4 weeks. Subsequently, the interval between each course was altered to every 3 weeks as the maximum tolerated dose (MTD) had not been reached at 45 mg/m^2 every 4 weeks and because animal experiments indicated that more frequent administration of lometrexol increased antitumour activity (Eli Lilly Co, unpublished results of G. Grindey, personal communication). Therefore lometrexol was subsequently administered every 3 weeks and doses escalated from 45 to 60, 78, 100, 130 mg/m^2 and 170 mg/m^2. No intra-patient dose escalation occurred.

Patients were evaluable for therapy-related antitumour activity if they had received two or more courses of therapy and disease measurements were recorded over at least an eight-week period from the first dose of therapy, with maintenance of a response for at least 1 month. A complete remission (CR) was defined as the disappearance of all tumour as assessed by physical examination and non-invasive investigations. A diminution by $> 50\%$ of the product of two diameters of a tumour was considered a partial remission (PR). Progression was indicated by the development of new lesions or an increase of 25% or more in the sum of the products of diameters of measurable lesions.

Pharmacokinetic studies

Lometrexol pharmacokinetics were determined in 24 patients receiving folic acid supplementation and lometrexol at all dose levels except 170 mg/m^2. The methodology used and the results obtained for the dose range 12–45 mg/m^2 has been published separately [12].

Results

Forty-three patients (23 females, 20 males) were recruited into the study who received a total of 99 courses. The pretreatment characteristics of the patients are shown in Table 1. The median age was 54 years (range 30–72 years). Thirty-five patients received at least 2 courses and were assessable for response and toxicity. Eight patients received only one course because of disease progression and were only assessable for toxicity. One patient who received only one course of lometrexol at 45 mg/m^2 every 4 weeks was ineligible due to the concurrent administration of allopurinol but was in any case assessed. The majority of patients had been pretreated, 31 of 43 patients (72%) had previous chemotherapy and 12 of 43 patients (28%) had also received prior radiotherapy.

Table 1. Patient characteristics

	No.
Total number of patients	43
Number of courses administered	99
Sex	
Females	23
Males	20
Age (yrs)	
Median	54
Range	30–72
Performance status (WHO)	
0	14
1	17
2	12
Tumour types:	
Colorectal carcinoma	14
Breast carcinoma	6
Non-small cell lung carcinoma	1
Malignant melanoma	6
Ovarian cancer	5
Pancreatic carcinoma	3
Renal cell carcinoma	3
Unknown primary carcinoma	3
Peritoneal carcinoma	1
Adenocortical carcinoma	1
Previous treatments	
Chemotherapy	31
Chemotherapy and radiotherapy	12

Myelotoxicity

Thrombocytopenia was the major toxicity observed in patients receiving ≥ 30 mg/m^2 lometrexol. Grade III–IV thrombocytopenia was observed in 7% (1/15), 14% (2/14), 7% (1/15), 20% (2/10) and 33% (3/9) of courses at doses of 30 and 45 mg/m^2 every 4 weeks, and 60, 100 and 130 mg/m^2 every 3 weeks, respectively (Table 2). However, the patient with grade IV thrombocytopaenia at 30 mg/m^2 developed ascites and paralytic ileus during treatment with lometrexol which might have effected the absorption of folic acid. One patient receiving lometrexol at 45 mg/m^2 every 4 weeks, who developed grade IV thrombocytopenia, also had bowel obstruction after receiving the second course. The two patients who developed grade IV thrombocytopenia (1 at 30 mg/m^2 and 1 at 45 mg/m^2 every 4 weeks) required hospital admission for leucovorin rescue, packed red blood cells and platelet transfu-

Table 2. Incidence of thrombocytopenia in patients treated with lometrexol given with folate supplementation

Dose level (mg/m^2)	Total patients	Total courses	Number of courses with WHO toxicity grade				
			0	I	II	III	IV
12 q 4wk	3	7	7	–	–	–	–
16 q 4wk	4	11	11	–	–	–	–
30 q 4wk	5	15	14	–	–	–	1[a] (2[b])
45 q 4wk	8	14	11	1(1)	–	1(1)	1(2)
45 q 3wk	3	8	6	1(4)	1(2)	–	–
60 q 3wk[c]	6	15	13	1(1)	–	1(2)	–
78 q 3wk[c]	3	8	8	–	–	–	–
100 q 3wk	5	10	8	–	–	2(1,2)	–
130 q 3wk	5	9	4	2(1,2)	–	1(3)	2(1,2)
170 q 3wk	1	2	1	1(2)	–	–	–

[a] Patient with ascites and paralytic ileus.
[b] The course number that toxicity developed.
[c] One patient each at 60 and 78 mg/m^2 received lometrexol for 6 and 4 courses, respectively, without experiencing thrombocytopenia.

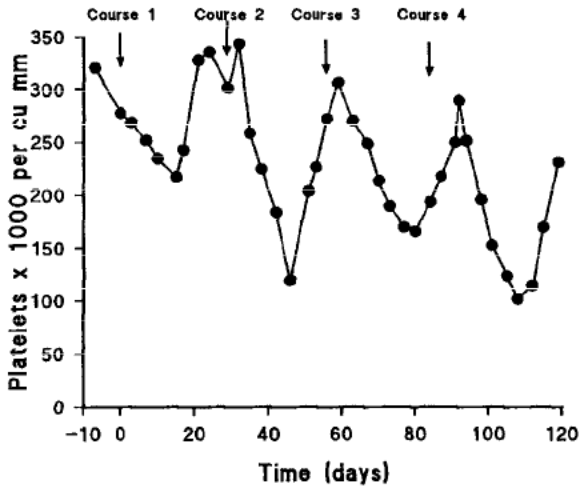

Figure 1. Lometrexol-induced cyclical reduction in platelet count in one patient receiving 4 courses of lometrexol at 30 mg/m^2 every 4 weeks. The timing of lometrexol administration is represented by arrows.

sions. Intravenous leucovorin (30 mg) was given every 6 hours for 12 days (patient at 30 mg/m^2 every 4 weeks) or 14 days (patient at 45 mg/m^2 every 4 weeks) until myelosuppression resolved. Although the majority of patients did not develop thrombocytopenia, a cyclical decrease in platelet counts following successive courses of lometrexol was observed in all patients and one example of a patient treated at 30 mg/m^2 is illustrated in Figure 1.

Leucopenia was infrequent and mild, the greatest toxicity observed in this study was grade IV in one patient (1/9 courses) and grade III in another patient (1/9 courses) at 130 mg/m^2 every 3 weeks. Overall, grade I–II leucopenia was observed on 27 of 99 courses (27%); however, grade III–IV toxicity was seen on only 4 of 99 courses (4%). No leucopenia greater than grade I was observed in patients receiving 12 or 16 mg/m^2 of lometrexol (18 courses).

Neutropenia was also infrequent and mild. No patient at 12 or 16 mg/m^2 lometrexol developed neutropenia (18 courses). Overall, grade I–II neutropenia was observed in 11% of courses (11/99) and grade III neutropenia developed in 2 patients (2/99 courses), one at 45 mg/m^2 every 4 weeks and one at 130 mg/m^2 every 3 weeks. Grade IV neutropenia with fever requiring intravenous antibiotics occurred in only one of 8 patients (1/14 courses) treated at 45 mg/m^2 every 4 weeks (after the second course of treatment). Grade IV neutropenia without fever was observed in one patient (1/10 courses) and 2 patients (2/9 courses) treated at 100 and 130 mg/m^2, respectively.

Anaemia became prominent in patients who received lometrexol for more than 2 courses. One patient treated at 30 mg/m^2, 2 patients at 45 mg/m^2 every 4 weeks and 2 patients at 100 mg/m^2 every 3 weeks (6/99 (6%) of the treatment cycles) developed grade III or IV anaemia. The three patients who developed grade III anaemia, one at 30 mg/m^2, one at 45 mg/m^2 and one at 100 mg/m^2, also had thrombocytopenia. The other patient at 45 mg/m^2 who developed grade III anaemia after the first course of treatment, which progressed to grade IV after receiving the second course, had upper gastrointestinal bleeding due to non-steroidal anti-inflammatory drug administration (diclofenac) and no thrombocytopenia. Therefore severe anaemia (grade III–IV) was observed on only 4% of courses (4/99), and mostly developed in thrombocytopenic patients. Mild anaemia (grade I–II) was observed on 46% of courses (46/99); however, frequent blood sampling for pharmacokinetic studies may have been partly responsible.

Gastrointestinal toxicity

Oral mucositis, manifested by diffuse erythema and small ulcers of the buccal mucosa, soft and hard palates and tongue, was observed in patients receiving $\geq$ 30 mg/m^2 lometrexol. Grade I to II oral mucositis occurred in 26 of 99 courses (26%). One of 6 patients at 60 mg/m^2 lometrexol every 3 weeks and the only patient treated at 170 mg/m^2 developed grade III toxicity after 2 courses of treatment (2 of 99 courses (2%)) (Table 3).

Table 3. Incidence of gastrointestinal toxicity in patients treated with lometrexol given with folate supplementation

Dose level (mg/m^2)	Total patients	Total courses	Number of courses with toxicity			
			Mucositis		Diarrhoea	
			I–II[a]	III[a]	I–II[a]	III[a]
12 q 4wk	3	7	0	0	0	0
16 q 4wk	4	11	0	0	2(18)	0
30 q 4wk	5	15	1(16)[b]	0	5(33)	1(6)
45 q 4&3wk	11	22	10(45)	0	1(4)	1(4)
60 q 3wk	6	15	1(6)	1(6)	1(6)	0
78 q 3wk	3	8	4(50)	0	0	0
100 q 3wk	5	10	3(30)	0	0	1(10)
130 q 3wk	5	9	7(77)	0	3(33)	1(11)
170 q 3wk	1	2	1	1	0	0
Total	43	99	26(26)	2(2)	12(12)	4(4)

[a] WHO toxicity grade.
[b] Percentage of courses associated with toxicity.

Diarrhoea was infrequent and mild (grade I to II on 12 of 99 courses (12%), Table 3); although grade III diarrhoea requiring intravenous fluid and folinic acid rescue did occur in 4 patients, one each at 30 and 45 mg/m^2 every 4 weeks, and 100 and 130 mg/m^2 every 3 weeks. However, the grade III diarrhoea observed in the one patient at 30 mg/m^2 after the second course of lometrexol may have been disease rather than lometrexol-related because this patient had paralytic ileus and ascites.

Mild nausea and vomiting (grade I to II) was observed in 37 of 99 courses (37%). Grade III vomiting was observed in only 3 patients (3 of 99 courses (3%)) and included the patient with ascites and paralytic ileus, and hence the toxicity may have been disease and not drug related.

Renal toxicity

In most patients renal function (GFR) was not altered by lometrexol treatment. However, 2 of 8 patients treated at 45 mg/m^2 every 4 weeks, experienced a decrease in GFR of $> 20\%$ following a single course of treatment. One of these two patients, who received a second course, had a further reduction from 65% of pretreatment following cycle 1 (164 to 107 ml/min), to 45% or pretreatment after cycle 2 (107 to 74 ml/min). In addition, one patient treated at 100 mg/m^2 every 3 weeks, had a reduction in GFR to 59% of pretreatment after cycle 2 (117 to 107 ml/min following cycle 1 and to 69 ml/min following cycle 2). However, both of these last two patients were receiving concomitant oral non-steroidal anti-inflammatory drugs (diclofenac and naproxen), which can cause renal dysfunction. According to the WHO classification of renal toxicity no patient in this study experienced renal toxicity, i.e. there were no increases in blood urea or creatinine to $> 1.25 \times$ upper limit of normal values.

Other toxicities

Two out of the six patients at 60 mg/m^2 every 3 weeks developed grade I and II muscular and joint pains a few days after treatment. No neurologic, cardiac, pulmonary, hepatic, cutaneous toxicities or alopecia were observed.

Efficacy of leucovorin in the reversal of lometrexol toxicity

Previous preclinical experiments have demonstrated that leucovorin can reverse the cytotoxicity of lometrexol [2, 13, 14]. Therefore leucovorin, initially at 30 mg i.v., was instituted for patients who developed $\geq$ grade III toxicities other than alopecia or nausea. Severe toxicity which needed leucovorin rescue occurred in 1 patient following the first course of lometrexol and in 2 patients following the second course. Thus leucovorin was given to a patient who developed grade III diarrhoea following the first course of lometrexol at 45 mg/m^2 every 4 weeks and the diarrhoea resolved within 48 hours of leucovorin administration. The two patients who developed grade IV thrombocytopenia after their second course of lometrexol, one at 30 mg/m^2 and one at 45 mg/m^2 every 4 weeks (the second patient having previously received leucovorin after the first course due to diarrhoea), were treated with 30 mg intravenous leucovorin every 6 hours for 12 days and 14 days, respectively, at which time myelosuppression resolved (Figure 2). However, as there was no clear evidence that leucovorin had reversed lometrexol toxicity, patients who developed severe toxicity at dose levels > 45 mg/m^2 were not treated with leucovorin.

Antitumour effects

An objective partial response was observed in one patient with metastatic breast cancer after her first course of lometrexol at 30 mg/m^2. The response was in a soft tissue lesion and was sustained for 48 days. One patient treated at 45 mg/m^2 every 3 weeks with metastatic breast cancer achieved a minor response, with an improvement in unmeasurable skin metastases and reduced dyspnoea for 10 weeks (but no significant

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