Phase I Study of the Antipurine Antifolate Lometrexol (DDATHF) with Folinic Acid Rescue

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ABSTRACT

Lometrexol (5,10-dideazatetrahydrofolic acid) is a new antifolate that is highly selective in inhibiting the key enzyme of purine synthesis, glycinamide ribonucleotide formyltransferase. The most promising preclinical features of lometrexol in animal models were its significant activity against a broad panel of solid tumors, the schedule dependency of its antitumor activity, and the availability of a rescue regimen with folinic or folic acid. In the present study, lometrexol was first given daily for 3 consecutive days, repeated every 4 weeks (part I). The occurrence of delayed myelotoxicity prompted the development of a rescue regimen with lometrexol given in a single dose on day 1, followed by oral folinic acid, 15 mg four times a day, from day 3 to day 5 (part II). Longer time intervals between administration of lometrexol and start of rescue were then evaluated (part III), and in the last part of the study (part IV), the maximum tolerated dose of single intermittent doses of lometrexol with folinic acid given from day 7 to day 9 was established. Sixty adult patients entered the study. In part I, the highest daily dose that could be safely given was 4 mg/m², for a total dose of 12 mg/m². Cumulative early stomatitis and delayed thrombocytopenia were dose limiting. The use of oral folinic acid made it possible to escalate the dose up to 60 mg/m², and the maximum tolerated dose was reached at this dose when folinic was given from day 7 to day 9, with anemia being the dose-limiting toxicity. A shorter time interval between lometrexol and folinic acid administrations (from day 5 to day 7) is recommended for Phase II evaluations to optimize the antitumor effect. Anemia was normochromic and macrocytic, possibly due to a deficiency of folic acid. One partial response of 8 months' duration was reported in a patient with epithelial cancer of the ovary, relapsing after cisplatin and alkylating agents. The use of folic acid as rescue, proposed on the basis of the available preclinical data, folinic acid was added because of cumulative anemia and thrombocytopenia, necessitating prolonged delays of treatment (4). In that study, on the basis of the available preclinical data, folinic acid was added in the presence of myelosuppression to hasten bone marrow recovery.

The aims of the present study were to evaluate MTD, pattern of toxicity, antitumor activity, and the pharmacokinetics of single-agent lometrexol administered for 3 consecutive days. Once achieved, the MTD of the single agent was evaluated in combination with folinic acid given at different doses and at different time intervals to define the safest and most rational schedule of rescue.

PATIENTS AND METHODS

Lometrexol, prepared as the disodium salt of the R diastereomer, was supplied by Lilly Research Laboratories (Indianapolis, IN) as an off-white crystalline solid in 20-mg vials. It was reconstituted with 0.9% saline for injection and administered as a rapid (30-s) iv. bolus. From May 1989 to October 1993, 61 adult patients with histological diagnoses of solid tumor, for whom therapies of proven efficacy were not available, entered this study. Eligibility criteria also included a WHO performance status ≤2, a life expectancy of ≥3 months, an

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3 The abbreviations used are: lometrexol, 5,10-dideazatetrahydrofolic acid; GAR, glycinamide ribonucleotide; MTD, maximum tolerated dose; mFBP, membrane folate-binding protein.
absolute neutrophil cell count \( \geq 2.0 \times 10^3/\mu l \), a platelet count \( \geq 100 \times 10^3/\mu l \), total bilirubin \( \leq 25 \mu mol/liter \), glutamic-oxaloacetate transaminase, or glutamic-pyruvic transaminase \( \leq 2.0 \) the upper limit of normal, creatinine \(<120 \mu mol/liter\), creatinine clearance \(>60 \text{ml/min}/1.73 \text{BSA} \), and normal RBC folate levels. Written informed patient consent was required as well. A need clearance >60 mi/mm/I .73 BSA, and normal RBC folate levels.

Since the daily schedule in animals was the most toxic, a daily \( \times 3 \) instead of the usual daily \( \times 5 \) was selected for the present study. The starting dose was fixed at 1.5 mg/m\(^2\)/day for a total dose per course of 4.5 mg/m\(^2\). This corresponded to less than one-third of the dose that was associated with acceptable toxicity in another ongoing Phase I trial (3). Cycles were repeated every 4 weeks, and the dose was increased by 50%, or in instances of toxicity, by 30% (part I).

Because of the occurrence of cumulative and delayed myelosuppression, precluding regular monthly treatments at doses \( \geq 4 \text{mg/m}^2/day \), the protocol was amended. In the subsequent part of the study, folinic acid rescue at a dose of 15 mg four times a day for 3 days, starting 2 days (day 3) after single doses of lometrexol, was routinely given. The highest dose given in part I was first investigated, and then the 30%-increased doses were evaluated (part II). The optimal schedule of treatment with folinic acid was subsequently defined by evaluating longer time intervals between lometrexol and the start of rescue (part III) to try to optimize the antitumor effect. The MTD of single intermittent doses of lometrexol in combination with folinic acid given at fixed times was defined in the last part of the study (part IV).

At parts I, II, and III, the entry of new patients was allowed only when the first patient had been followed for at least 3 weeks, and three patients had to complete the first cycle before subsequent patients could be treated at the higher dose. In part IV, at least three patients had to complete two courses of treatment without serious toxic effects before dose escalation. In the two last parts, rescue with folinic acid was prolonged for 2 days if stomatitis of more than or equal to grade 2 with no sign of recovery was still present after the first 3 days (day 5) of rescue. A full blood count was done twice a week, and biochemistry and urinalysis were done once a week. Toxicity and response were evaluated according to WHO criteria (5).

A high-performance liquid chromatography with fluorescence and UV detection for the measurement of lometrexol in plasma and urine, respectively, was used for pharmacokinetic analyses in the patients treated in part IV of the study (6). Some of the pharmacokinetic results have been already reported in a separate paper (7).

**RESULTS**

Of 61 patients treated, 60 had follow-up periods of at least 3 weeks and were evaluable for toxicity. One patient with hepatocellular carcinoma died of tumor progression 16 days after the first dose of lometrexol. The schedule of treatment and the number of patients who entered the subsequent parts of the study are reported in Table 1. All but five of the patients were pretreated with chemotherapy, and 33% of them had been given radiotherapy. The most frequent tumor types were colon (26% of patients), ovary (23%), and non-small cell lung cancer (16%).

**Toxicity.** The administration of lometrexol on 3 consecutive days was associated with dose-limiting, cumulative mucositis, mainly stomatitis, appearing in the first week after the treatment, and cumulative, prolonged thrombocytopenia (Table 2). Four mg/m\(^2\) was the highest daily dose that could be safely given, but the occurrence of a double platelet count nadir, which was already present at 3 mg/m\(^2\), impeded the administration of therapy on a monthly basis. Platelet counts followed a distinctive pattern; they started to decrease 2 weeks after the treatment, achieved a first nadir in the following week, recovered to baseline values in week 4, and decreased again to achieve a second nadir, all within 5 weeks after treatment. This pattern was observed in all four patients treated at 4 mg/m\(^2\) and was even more pronounced in the two patients who received a second cycle of treatment.

A rescue with 15 mg four times a day of oral folinic acid from day 3 to day 5 was then evaluated (part II; Table 1).
Lometrexol was administered on day 1 as a single i.v. bolus at a dose of 12 mg/m² (three patients and 5 evaluable cycles), 16 mg/m² (three patients and 12 cycles), and 21 mg/m² (three patients and 6 cycles). Because of the lack of myelosuppression and stomatitis, even after repeated administrations at the highest dose (21 mg/m²), the start of rescue was progressively delayed, first until day 5 (five patients and 9 courses) and then until day 7 (four patients and 11 courses) (part III). While only one of the five patients in the former group suffered from grades 2 and 3 anemia after the third and fourth cycles, three of the four patients in the latter group developed cumulative anemia. The lowest hemoglobin value occurred 4 weeks after the treatment and recovered within 1 week. Stomatitis occurred in only one patient in the latter group, on day 5 of the first cycle, was only partially controlled by the earlier start of folinic acid during the subsequent cycles, and was never associated with anemia.

In addition, at the higher doses of 30 and 45 mg/m², the same schedule of rescue with folinic acid from day 7 to day 9 prevented the early appearance of stomatitis, even after repeated administration (Table 3). One patient developed grade 3 stomatitis after the third and fourth doses of 30 mg/m², associated with grades 2 and 3 anemia, respectively, and grade 4 thrombocytopenia (see below). Stomatitis was recurrent and reproducible, appearing between day 5 and day 7, and recovering within 3 days if of grade 1 but lasting up to 7–10 days if of grade 3. Once manifested, stomatitis was not ameliorated by the start of folinic acid rescue.

Anemia was cumulative and delayed, with a median time to nadir of 28 days (range, 13–57 days). It was also long lasting, with a median time to recovery of 39+ days (range, 21–81+). The use of half doses of folinic acid (7.5 mg four times a day) was more frequently associated with stomatitis but not with anemia, even after the first administration of 30 mg/m² (Table 3).

Increasing the dose from 45 to 60 mg/m² did not result in a higher incidence of stomatitis and anemia when folinic acid was given from day 5 to day 7 but was associated with an overall higher incidence of anemia when folinic acid was given from day 7 to day 9, as at the lower dose levels (Table 3). The median time to hemoglobin nadir and recovery was 16 days (range, 11–44 days) and 30+ days (range, 22–51+ days), respectively.

At all dose levels, anemia was mainly normochromic and macrocytic with no signs of hemolysis. Lometrexol seemed to affect mainly RBC counts, with only sporadic episodes of thrombocytopenia. One patient with cancer of the ovary pretreated with cisplatin, cyclophosphamide, and taxol developed a platelet count of 9 × 10³/µl 31 days after the fourth dose of 30 mg/m² and had bone marrow aspiration performed because of the occurrence of severe and cumulative anemia (see below). One patient with renal cell carcinoma, pretreated with cisplatin, vinblastine, α-IF, and radiotherapy, had anemia and grade 3 thrombocytopenia 6 weeks after the second dose of 30 mg/m² and underwent a bone marrow aspiration (see below). Thrombocytopenia grade 3 was also observed in two heavily pretreated patients, after the second cycle at 45 mg/m² in one and after the first cycle at 60 mg/m² in the other. In the latter case, the patient had received prior 5-fluorouracil, folinic acid, nitrosoureas, and mitomycin C. Rescue was prolonged beyond the usual 3 days in only 8 cycles at 45 and 60 mg/m²; therefore, its impact in hastening marrow recovery could not be assessed.

**Appearance of Bone Marrow and Blood Cells.** Bone marrow aspiration was performed in four patients suffering from grade 3 prolonged anemia. Bone marrow smears showed either decreased or increased erythropoiesis with megaloblastic alterations of the mature forms, increased or normal megakaryopoiesis with larger megakaryocytes, and increased myelopoiesis with nuclear-cytoplasmic dissociation of granulocyte precursors. Overall, these findings support a diagnosis of megaloblastic anemia.

In the peripheral smears, anemia was normochromic, with mean corpuscular volumes ranging from 92 to 102 µm³ and reticulocytopenia in all patients. In three patients, a few hypersegmented neutrophils were present. In all patients, lactate dehydrogenase was within normal values. Serum vitamin B₁₂ was

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**Table 3** Stomatitis and anemia after repeated administrations of lometrexol with folinic acid* (part IV)

<table>
<thead>
<tr>
<th>Lometrexol (mg/m²)</th>
<th>No. of patients</th>
<th>No. of cycles</th>
<th>Stomatitis</th>
<th>Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>30*</td>
<td>6</td>
<td>Initial 6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent 7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>30*</td>
<td>7</td>
<td>Initial 7</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent 5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>45*</td>
<td>6</td>
<td>Initial 6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent 6</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>60*</td>
<td>6</td>
<td>Initial 6</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent 6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>60*</td>
<td>6</td>
<td>Initial 6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subsequent 7</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* Folinic acid, 15 mg four times a day, days 7–9.
* Folinic acid, 7.5 mg four times a day, days 7–9.
* Folinic acid, 15 mg four times a day, days 5–7.
* Folinic acid, 15 mg four times a day, days 7–9.
normal, and RBC folates were normal or increased, due to a cross-reactivity with lometrexol. Peripheral blood cell counts rapidly improved after the start of folinic acid supplementation.

**Antitumor Activity.** One patient with a pelvic and retroperitoneal recurrence of clear cell carcinoma of the ovary, pre-treated with cisplatin, alkylating agents, and radiotherapy, had a partial response with lometrexol at the dose of 16 mg/m². Her treatment was continued until tumor progression, for a total of 6 courses. No other objective responses were reported.

**DISCUSSION**

Lometrexol is the first clinically evaluated antifolate that acts primarily through a selective inhibition of GAR formyl-transferase, the first folate-dependent enzyme in the de novo synthesis of purine. A daily treatment was initially selected for this Phase I study because of the greater antitumor activity reported with this schedule in animal models. Although treatment was administered for only 3 days, this regimen proved to be too toxic, thus confirming the toxicology data and discouraging further clinical evaluation. Early stomatitis was dose limiting at 12 mg/m² total dose, whereas delayed thrombocytopenia did not allow repetition of the therapy at monthly intervals. Stomatitis of grades 3–4 occurred in the first week of treatment in 25% of patients after the first cycle and in all patients after the second.

The development of a rescue regimen was the subsequent obligatory step, and folinic acid was selected on the basis of the available preclinical data which showed that: (a) the cytotoxicity of lometrexol in cell culture can be completely reversed by the coinfusion of high but not low doses of folinic acid (2); and (b) toxicity of lometrexol in dogs can be partially rescued by repeated doses of folinic acid starting up to 10 days after its appearance (2). The schedule for folinic acid was empirically drawn from the one currently used for methotrexate, with a daily dose of 15 mg four times a day. The start of folinic acid was progressively delayed from day 3 to day 5 and day 7.

Rescue with folinic acid could also avert severe stomatitis after repeated administrations of the highest dose of 60 mg/m². A daily dose of 15 mg four times a day seemed to be the optimal one for the rescue of stomatitis since the same dose of lometrexol administered with of 7.5 mg four times a day resulted in a higher overall incidence of stomatitis, even after the first administration.

The other main side effect of lometrexol was anemia, which was cumulative, delayed, and long-lasting at all dose levels. Anemia was the main criteria in the definition of MTD, which was determined to be 60 mg/m² of lometrexol with folinic acid from day 7 to day 9. With this regimen, grade 2 anemia was observed in 30% of patients after the initial course and 57% after subsequent courses, with a median time to recovery of more than 4 weeks. The recommended dose for Phase II studies was 60 mg/m² of lometrexol with folinic acid given from day 5 to day 7. This schedule resulted in grade 2 anemia being reported in less than 20% of patients, even after repeated administrations.

Bone marrow findings were consistent with a diagnosis of megaloblastic anemia, the mechanism of which is reported to be damage of the DNA synthesis process, with unbalanced growth and premature cell death, varying from cell to cell and from cell series to cell series, usually more evident against erythrocyte than granulocyte precursors. In view of the absence of a vitamin B₁₂ deficiency and lack of neurological symptoms, as well as the observation of a clinical response to folinic acid, this megaloblastic anemia was probably caused by a folic acid deficiency. The effect of folic acid deficiency was more pronounced on RBCs and platelets than on neutrophils, and it cannot be excluded that a particular sensitivity of erythrocyte precursors and megakaryocytes might be due to other factors, such as cellular folypolyglutamate synthetase, which are relevant for lometrexol cytotoxicity. To our knowledge, however, no data are available on this point.

From an empirical point of view, the results of this study show that the use of folinic acid can allow the repeated administration of lometrexol in doses higher than in the previous studies. The objection that folinic acid can also negate the antitumor effect of lometrexol is, at least partly, counteracted by the report of an objective response in one patient with cancer of the ovary who received a dose much lower than that recommended for clinical studies. It cannot be excluded, however, that folinic acid can mitigate the antitumor effect of lometrexol, except for tumors that exhibit a selective mechanism of drug uptake such as ovarian cancer, which expresses high levels of mFBP (8, 9). It should be pointed out that other factors, including reduced folate carriers and folypolyglutamate synthetase, are important determinants of sensitivity to lometrexol (10).

It was observed that low doses of folic acid can prevent the toxicity but not the antitumor activity of lometrexol in mice (11) and that the antitumor activity of lometrexol was dependent upon dietary folic acid intake in C,H mice (12, 13). Therefore, a Phase I trial of lometrexol with a folic acid supplement, given 7 days before and 7 days after lometrexol, was started (14). In this ongoing study, the MTD has not been reached at 170 mg/m², and a further dose escalation is planned.

Concomitantly with clinical evaluations, many preclinical studies have been carried out to elucidate the mechanism by which folinic and folic acids counteract lometrexol-induced toxic effects and to provide information that could be relevant for a rational clinical development of this antifolate. Many aspects of the cellular pharmacology of lometrexol have been clarified. Lometrexol has a high affinity for mFBP and, to a lesser extent, for the reduced folate carrier, the most relevant one for folic and folinic acids. Once in the cell, lometrexol is transformed into polyglutamated compounds, which are more potent inhibitors of GAR formyltransferase than the monoglutamated parent compound, and these are more effectively retained in tumor cells. A decrease in folypolyglutamate synthetase has been shown to represent one of the mechanisms of resistance in human leukemia-resistant sublines (15). The expression of mFBP is different in neoplastic and normal tissues and can be affected by folic acid concentrations. All these aspects, as well as some of the folate metabolism, have been shown to be involved in vitro and partly in vivo in the modulation of the toxicity and activity of lometrexol by folinic or folic acids.

Erba et al. (16) showed in a human ovarian carcinoma cell line SW626 that low folic acid concentrations do not block the cytotoxicity of lometrexol. In fact, cells growing in the presence
of 0.22 μM folic acid, which is at the upper end of folate plasma levels after folic acid supplementation (7), had an IC50 for lometrexol of 0.0024 μg/ml, which is a lower drug concentration than that found during the 3 days following a lometrexol dose of 45 mg/m2. In the same study (16), concomitant exposure to folic acid completely reversed the cytotoxicity of lometrexol, while administration of folic acid 24 h after lometrexol did not affect the cytotoxicity of the antifolate. These results, in agreement with those of this Phase I study, suggest that the interval between lometrexol administration and the start of folic acid should be several days to avoid decreasing the antitumor activity of lometrexol.

As already reported (7), the pharmacokinetics of lometrexol was studied in seven patients treated in part IV at 45 or 60 mg/m2. In the majority of patients, the plasma elimination followed a triexponential compartment model with a t1/2-β of 169 ± 51 min and a t1/2-γ, where measurable, of 2593 ± 1671 min. After the administration of 45 mg/m2, a plasma concentration of 0.15 μg/ml was maintained for up to 72 h (7). This is higher than the concentration of 0.024 μg/ml shown in in vitro studies to be associated with >90% inhibition of clonogenicity with a time interval of 96 h between lometrexol and the start of folic acid, cytotoxic concentrations are achieved and maintained for an adequate period of time.

It is still difficult to determine whether folic or folic acid should be given in combination with lometrexol. We would like to point out that the continuous exchange of results among preclinical clinical investigations has resulted in a more rational and successful conduct of the Phase I studies, such that the clinical use of both folic and folinic acids is supported by preclinical in vitro and in vivo data. Supplementation with folic acid, on the basis of convincing experimental results, has allowed the repeated administration of higher doses at less cost. Supplementation with folic acid will be implemented in the early clinical evaluation of the thiophene analogue of lome-}

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