Clinical Pharmacokinetics of the Antipurine Antifolate (6R)-5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (Lometrexol) Administered with an Oral Folic Acid Supplement

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ABSTRACT

(6R)-5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (lometrexol) is an antipurine antifolate which selectively inhibits glycaminamide ribonucleotide formyltransferase. Lometrexol pharmacokinetics were evaluated in 17 patients (32 courses) as part of a Phase I study in which folic acid supplementation was used to improve tolerance to the drug, its clinical utility being previously limited by severe cumulative toxicity. Lometrexol was administered as an i.v. bolus every 4 weeks at a starting dose of 12 mg/m², with subsequent interpatient dose escalation to 16, 30, and 45 mg/m². p.o. folic acid (5 mg/day) was given for 7 days before and 7 days after lometrexol administration. The disposition of total lometrexol in plasma was best described by a biexponential model for data acquired up to 12 h after drug administration, although triexponential plasma pharmacokinetics were often found to give a more adequate description when data were available at later time intervals (24 h and greater). Mean plasma half-lives (± SD) for model-dependent analysis were \( t_{1/2} \) 19 ± 7 min, \( t_{1/2} \beta \) 256 ± 96 min, and \( t_{1/2} \gamma \) (where measurable) 1170 ± 435 min. Lometrexol area under plasma concentration versus time curve was proportional to the dose administered. Moderate plasma protein binding of lometrexol was evident (78 ± 3%) with an inverse linear relationship between fraction of unbound lometrexol and the concentration of serum albumin. The volume of distribution at steady state was 4.7 and 15.8 l/m². Renal elimination of lometrexol, studied in 19 patients (21 courses), was considerable, accounting for 56 ± 17% of the total dose administered within 6 h of treatment, and 85 ± 16% within 24 h of treatment. These recoveries of unchanged lometrexol indicate that the drug does not appear to undergo appreciable systemic metabolism at the range of concentrations studied.

Lometrexol pharmacokinetics were also examined in seven patients who received 45 or 60 mg/m² lometrexol as part of a separate study of the drug given with folic acid rescue 5–7 days after treatment. No marked differences were evident in lometrexol plasma half-lives, plasma clearance, or the extent of plasma protein binding, indicating that there is not a pronounced pharmacokinetic interaction between lometrexol and folic acid.

INTRODUCTION

Lometrexol is a folate analogue which selectively inhibits GAR formyltransferase, an enzyme essential for de novo purine biosynthesis (1, 2). This antipurine antifolate exhibits a broad spectrum of antitumor activity in murine and human xenograft tumor models, in which the established antifolate methotrexate demonstrates little or no effect (3).

In early Phase I clinical studies with lometrexol, significant clinical toxicity was evident, characterized by severe mucositis and myelotoxicity (thrombocytopenia and leucopenia), which limited drug administration to only one or two courses (4–7). These toxicities were unexpected, occurring at drug concentrations which were approximately one hundredth of the 10% lethal dose in mice (8). A number of clinical responses were documented, including activity against malignant fibrous histiocytoma (5), non-small cell lung cancer, breast cancer, and colonic adenocarcinoma (7), which stimulated studies aimed at the pharmacological amelioration of lometrexol toxicity. Experimentation in mice revealed that the therapeutic index of lometrexol was highly dependent on dietary folic acid intake (9, 10), and suggested that folic acid administration could reduce toxicity, without abating antitumor activity. To enable the development of a tolerable and effective schedule for the routine clinical use of lometrexol, a Phase I study was initiated in which 5 mg folic acid/day were given for 7 days before and after lometrexol administration. After 7 days, this dose of folic acid resulted in an increase in plasma folate levels from 3 to 64 ng/ml to 6 to 180 ng/ml in the patients studied.

The Phase I study of lometrexol with folic supplementation provided an opportunity, for the first time, to conduct detailed clinical pharmacokinetic studies with lometrexol; a comprehensive pharmacological examination in humans being previously prohibited by the lack of clinical utility and availability of a suitable assay. The principal objectives of this...
clinical pharmacokinetic study were: (a) to determine the plasma pharmacokinetics in patients receiving multiple courses of lometrexol, and thereby define the relationship between lometrexol AUC and dose, and intra- and interpatient variability in AUC; (b) to investigate the extent of lometrexol plasma protein binding; (c) to measure urinary excretion of lometrexol; and (d) to evaluate the effect of folic acid supplementation on lometrexol pharmacokinetics to determine whether the improved tolerance of lometrexol produced by folic acid administration is a consequence of a pharmacokinetic interaction.

MATERIALS AND METHODS

Patient Eligibility. Patients eligible for this study had a histologically documented malignant solid tumor, which was either refractory to established therapies, or for which no standard therapy existed. All patients had a predicted life expectancy of at least 12 weeks, and had recovered from the toxic effects of previous treatment before entering into the study, i.e., they had not received any major therapy or investigational drug for at least 4 weeks (6 weeks if prior therapy included chemotherapy with a compound known to have delayed toxicity, e.g., a nitrosourea). Exclusion criteria included factors which could have interfered with lometrexol disposition/toxicity or folic acid absorption and comprised: (a) concomitant medication with probenecid, trimethoprim, co-trimoxazole, pyrimethamine, prednisolone, anti-inflammatory, ulcerative bowel disease or malabsorption syndrome.

All patients were required to have adequate organ function prior to treatment, with hepatic function characterized by bilirubin levels of <25 µmol/liter, and renal function by a creatinine measurement of <120 µmol/liter and a 51Cr-EDTA clearance of >50 ml/min. Informed written consent was given according to local regulatory requirements.

Study Design. Folic acid (Approved Prescription Services Ltd., Leeds, United Kingdom) was given daily as a single 5-mg tablet for 7 days before and 7 days after lometrexol administration at 4-week intervals. Lometrexol (Lilly Research Centre, Surrey, United Kingdom) was reconstituted in 0.9% (v/v) saline and administered as a rapid i.v. bolus over 0.5–1.0 min 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 an additional 24 h after drug administration to ensure that acute toxicity was not apparent. The performance status of patients was assessed at least once a week, for a period of 4 weeks, following lometrexol therapy.

The trial design required three patients, previously untreated with lometrexol, to be treated at each dose level. The first patient entered at each dose level was followed up for 3 weeks before the next patient was entered. At least two patients per dose level received two courses before dose escalation. Toxicities were evaluated according to the 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 judgment of the investigator with approval of the Medicines Control Agency (London, United Kingdom) and the Local Ethics Committee. The starting dose of lometrexol was 12 mg/m², with subsequent escalation to 16, 20, and 45 mg/m². Dose escalation increments were determined by clinical experience at the previous dose level and by data from a parallel study of lometrexol given with folic acid (11). No intrapatient dose escalation occurred.

Pharmacokinetic Studies. Lometrexol pharmacokinetics was determined in 17 patients (32 courses) receiving folic acid supplementation and in an additional 7 patients (7 courses) who did not receive folic acid. Plasma samples from patients receiving lometrexol without folate supplementation were kindly provided by Drs. C. Sessa and F. Cavalli (Ospedale San Giovanni, Bellinzona, Switzerland), who were responsible for an alternative Phase I study that involved folic acid administration (15 mg every 6 h for 12 doses), starting 5–7 days after treatment with lometrexol (11).

Blood samples were collected by venipuncture into vacutainer tubes placed on ice and containing the sodium salt of EDTA as an anticoagulant, and were taken before treatment and at 5, 15, 30, and 45 min and at 1, 1.5, 2, 4, 6, 8, 12, and 24 h, and in some patients at 48, 72, and 96 h, after lometrexol administration. Samples were immediately centrifuged (1000 × g, 8 min, 4°C), and plasma was removed by aspiration with a Pasteur pipette. Plasma was stored at −20°C prior to analysis.

The plasma lometrexol concentration was measured by the HPLC method of Wedge et al. (12), which uses derivitization and fluorescence detection. Briefly, patient samples were thawed at room temperature and diluted to 1 ml with control human plasma (Red Cross Transfusion Service, Newcastle-upon-Tyne, United Kingdom) to contain 10–250 ng/ml lometrexol. Samples were further diluted (1:1) with aqueous formic acid [1% (v/v); pH 3.7] containing 100 ng C14-desmethylene lometrexol (Lilly Research Centre) as an internal standard. Following rotary mixing and centrifugation, each sample was subjected to solid-phase extraction using a C8 (1 cm) Bondelut cartridge (Analytichem International, Harbour City, CA). Eluted samples were evaporated to dryness using a Speedvac concentrator (Savant Ltd., Farmingdale, New York) and reconstituted in 13% (v/v) aqueous formic acid. Oxidation of lometrexol and the internal standard was achieved by incubation (37°C, 90 min) with a suspension of manganese dioxide (0.2 mg/ml) in water and terminated by the addition of a 1:1 mixture of 5 N NaOH and 1% (w/v) ammonium carbonate (pH 5) to samples on ice. Samples were centrifuged (13,000 × g, 10 min), and 100 µl of the supernatant were analyzed chromatographically. Chromatographic analysis was achieved using an Apex II (C18, 3 µm; 150 × 4.6 mm) analytical column (Jones Chromatography, Hen-goed, Glamorgan, South Wales, United Kingdom) and a mobile phase of 12% (w/v) acetonitrile in 1% (v/v) aqueous acetic acid (pH 5) containing tetramethylammonium hydrogen sulfate (0.171 g/liter) as an ion pair reagent. Elution was isocratic, at a flow rate of 1 ml/min, and analyte measurement was by fluorescence detection (Emax, 325 nm; Einf, 450 nm).

To assess intraassay variation, each assay was calibrated using a five-point standard curve of duplicate lometrexol standards in the range 10–250 ng/ml, prepared in control human plasma, and extracted/analyzed at the same time as patient samples. Quantitation was achieved using internal standardization by a comparison of peak height ratios, with peak heights being quantified using Minichrom Software (VG Data Systems Ltd., Altrincham, Cheshire, United Kingdom). All calibration
curves were linear ($r^2 > 0.997$), the lower limit of determination of the assay was 10 ng/ml, and the intra-assay CV at nominal concentrations of 10, 50, and 250 ng/ml was always <8.5%. In addition, three lometrexol quality assurance samples (10, 50, and 250 ng/ml) prepared every 2–3 months in bulk, were assayed in triplicate to assess the interassay CV, which was always <6%.

**Pharmacokinetic Parameters.** Plasma lometrexol data were analyzed using both model-independent and model-dependent analyses. In model-independent analyses, the AUC was calculated using the log trapezoidal rule (13) with extrapolation to infinity, using the terminal phase rate constant calculated by the compartmental analysis. For model-dependent analyses, a biexponential or triexponential equation was fitted to the concentration versus time data using a nonlinear-weighted least-squares parameter estimation program (ADAPT II, kindly provided by Dr. S. D. Z. D’Argenio and A. Schumitzky, Biomedical Simulations Resource, Los Angeles, CA). Data were weighted as the reciprocal of the estimated variance, where the SD of the output was assumed to be proportional to the estimated concentration (constant CV). The model providing the best fit to each data set was determined using the precision of the parameter estimates and consideration of the Akaike information criterion (14). The parameters derived were used to calculate model-independent (AUC, $C_{\text{T1/2}}$, $V_d$) or model-dependent (AUC, $C_{\text{T1/2}}$, $t_{1/2g}$, $t_{1/2b}$, and $t_{1/2y}$) pharmacokinetic parameters (13, 15).

**Plasma Protein Binding.** Lometrexol plasma protein binding was determined using $^{14}$C-lometrexol (specific activity, $13 \mu Ci/mg$), which was kindly provided by Dr. M. D’Incalci (Istituto Mario Negri, Milano, Italy). This compound was radio-labeled at the carbonyl group of the benzyl moiety, and had a radiochemical purity of >88%, as determined by HPLC. Each patient plasma sample (1 ml) was spiked with 3.5 μg $^{14}$C-lometrexol, rotary mixed, and an aliquot (100 μl) was removed. The remainder was then subjected to ultrafiltration using an Amicon Centricon Micropartition Unit (Amicon Corp., Upper Mill, Stonehouse, Gloucestershire, United Kingdom) and centrifugation (1000 $\times$ g, 10 min, 4°C), after which an aliquot (100 μl) of ultrafiltrate was removed. $^{14}$C-Lometrexol in the ultrafiltrate and prefiltered plasma was determined by liquid scintillation counting, and the ratio was used to calculate the percentage of unbound lometrexol. Nonspecific binding of $^{14}$C-lometrexol to the filter was determined to be <4% by ultrafiltrating solutions of $^{14}$C-lometrexol in PBS (0.1 M, pH 7.4; 1 ml) and was therefore ignored. Samples of plasma from each patient, taken at <1 h and at 12–120 h after lometrexol administration were examined to assess any potential concentration dependency of lometrexol plasma protein binding, but no significant differences in binding were found ($P = 0.11$, paired $t$-test). The mean of each pair of analyses was therefore used to: (a) relate the unbound fraction of lometrexol with serum protein and plasma protein concentrations and (b) calculate the unbound $V_d$, (15).

**Urinary Excretion.** Urinary excretion was studied in 13 patients (14 courses) for which there was evaluable plasma pharmacokinetics available, and in an additional 6 patients (7 courses) for which plasma pharmacokinetics was not measured. Urine samples for lometrexol analysis were collected for 24 h after drug administration at 6-hour intervals, and 20 ml aliquots were stored at −20°C prior to analysis. Samples were thawed at room temperature and diluted in control urine to ensure that the lometrexol concentration would be between 2 and 25 μg/ml (i.e., the dilution factor used was estimated according to the dose of lometrexol administered and the volume of urine produced in a 6-h period). The method for sample preparation was as described for plasma samples, except $^{14}$C-desmethylene lometrexol was added at a concentration of 15 μg/ml, and evaporated samples were not oxidized, but resuspended in 150 μl of the mobile phase and 50 μl were analyzed chromatographically. Chromatographic analysis (12) involved a Spherisorb C6 (5 μm; 150 × 4.6 mm) analytical column (Jones Chromatography) and isocratic elution with a mobile phase of 13% (w/v) acetonitrile in aqueous phosphoric acid [1% (v/v), pH 3.2] at a flow rate of 1.5 ml/min. Each assay included a five-point standard curve (duplicate samples within the range 0.2–10 μg/ml), and quality assurance standards at 0.2, 2, and 10 μg/ml were assayed in triplicate. The intra-assay and interassay CVs for these assays were found to be <2% and <3%, respectively. Lometrexol excretion in urine was expressed as a percentage of the administered dose, and renal clearance was calculated as the ratio of the total amount excreted and the AUC.

**Statistical Methods.** All values expressed with a margin of error represent the mean ± SD. Levels of significance were calculated using Student’s $t$ test, where $P < 0.05$ was considered indicative of a significant difference between groups. The relationship between lometrexol dose and pharmacokinetic parameters was assessed by linear regression analysis and/or a Spearman rank correlation.

**RESULTS**

**Pharmacokinetics of Lometrexol Administered with a Folic Acid Supplement.** The characteristics of the patients studied are shown in Table 1, and plasma lometrexol concentration-time profiles from representative patients receiving 12, 16, 30, or 45 mg/m² lometrexol are shown in Fig. 1. Data collected within the first 12 h of lometrexol administration were found to be best described by a biexponential equation when evaluated by compartmental analysis. However, at doses of 30 and 45 mg/m² lometrexol and where data were available at later
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set all had coefficients of determination (r^2) of >0.92, i.e., 0.99 median (range, 0.92–0.99).

Pharmacokinetic parameters are shown in Table 2 with parameters for patients receiving more than two courses of lometrexol being represented as the mean and SD of all courses studied in that patient. Model-dependent analysis of lometrexol pharmacokinetics resulted in a t1/2α and t1/2β of 19 ± 7 min and 256 ± 94 min, respectively, and a t1/2γ (where measurable) of 1170 ± 435 min.

Calculation of both lometrexol AUC and ClTOT by either a model-independent or model-dependent analysis was not found to result in any significant difference (P = 0.14 and P = 0.33, respectively, paired t test), and there were strong linear relationships for both parameters for both analyses (r > 0.98).

Model-independent analysis indicated that lometrexol AUC was linearly related to dose (r = 0.88, P < 0.001; Fig. 2), i.e., plasma clearance of lometrexol was not dose dependent. However, two patients had a lometrexol AUC and ClTOT which differed markedly from that observed in other patients treated at the same dose level. One patient (patient 12) receiving 30 mg/m² lometrexol had a consistently greater AUC than for others receiving the same dose, i.e., 2.08–2.28 mg/ml · min compared with 1.38 and 1.50 mg/ml · min (Table 2 and Fig. 2). Reduced lometrexol clearance in this patient may have been due to an underlying early left ventricular failure, which could have decreased cardiac output and thereby reduced tissue perfusion, combined with a relatively low pretreatment GFR of 75 ml/min, which may have influenced the renal excretion of lometrexol. In contrast, at a dose of 45 mg/m² lometrexol, one patient (patient 19) had a much lower AUC than was measured in others (1.27 mg/ml · min compared with values of 1.60–2.20 mg/ml · min), with a correspondingly higher ClTOT (35.8 ml/min/m² compared to values of 23.1–27.7 ml/min/m²). This may be attributable to an unusually large lometrexol Vd, in this patient of 15.8 liters/m², which in turn could have been caused by the presence of a bilateral pleural effusion, combined with a high pretreatment GFR of 165 ml/min which would have promoted extensive renal excretion of lometrexol.

No consistent change in the model-independent AUC was evident following more than one course of lometrexol, with the possible exception of three patients receiving 45 mg/m² lometrexol every 4 weeks (patients 13, 16, and 17), in whom small increases in AUC after the second and third courses of lometrexol were observed. In patients receiving more than three courses of lometrexol, no consistent change in clearance could be found, i.e., the intrapatient CV of plasma clearance was 14% (median; range, 3–21%; n = 5).

The lometrexol Vd, varied between 4.7 and 15.8 l/m², and the unbound lometrexol Vd, calculated from measurements of lometrexol plasma protein binding (see below), varied between 18.1 and 67.1 l/m². The larger Vd, of two patients receiving 45 mg/m² lometrexol may be attributed to the fact that both patients had pleural effusions. Even if data from these two patients are excluded, rank correlations are observed between dose level (mg/m²) and both Vd, (r = 0.84, P < 0.001) and unbound Vd, (r = 0.66, P < 0.01).

**Plasma Protein Binding of Lometrexol.** The percentage of unbound lometrexol in plasma was 22 ± 3%, indicating plasma protein binding of 78 ± 3%. Protein binding determined at two concentrations of lometrexol (3.5–4.7 and 7.7–21.1 μg/ml) revealed that binding was not concentration dependent (P = 0.11, paired t test). An inverse linear relationship was apparent between the lometrexol binding and serum albumin concentration (r = 0.88; P < 0.001; Fig. 3). The relationship between unbound lometrexol and total serum protein was not found to be significant (r = 0.38; P > 0.05), when one patient with particularly low total serum protein (55 g/liter) was removed from the analysis.

**Urinary Excretion of Lometrexol.** The 0–24-h cumulative urinary excretion data (Fig. 4) for 19 patients (21 courses) indicated that the major elimination route for lometrexol was renal excretion, with 85 ± 16% of the administered dose being excreted within 24 h of drug administration and 56 ± 17% within the first 6 h. Urinary excretion studied in two patients who received two consecutive courses of lometrexol did not reveal any consistent change in excretion following the second course of treatment. Two patients had 0–24 h lometrexol urinary recoveries of >100% of the administered dose, which was likely to be due to inaccuracies in the measurement of urine.
Table 2  Pharmacokinetic parameters for lometrexol administered with folic acid supplementation

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<th>Patient/course</th>
<th>Dose level (mg/m²)</th>
<th>t_{1/2} (min)</th>
<th>t_{1/2} (min)</th>
<th>t_{1/2} (min)</th>
<th>AUC (mg/ml/min)</th>
<th>Total clearance (ml/min/m²)</th>
<th>Renal clearance (ml/min/m²)</th>
<th>Vd (liters/m²)</th>
<th>Unbound Vd (liters/m²)</th>
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<td>45</td>
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<td>147</td>
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<td>15.6 ± 0.4</td>
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Mean ± SD (n = 17) 19 ± 7 256 ± 96 1170 ± 435 22.6 ± 4.7 21.2 ± 7.5

Fig. 2 Relationship between lometrexol dose and AUC calculated from noncompartmental analysis. Data are given in Table 2, and the line is that generated by linear regression analysis.

Fig. 3 Relationship between serum albumin concentration and the percentage of lometrexol which was unbound to total plasma protein. The line is that given by linear regression analysis.

The 0–24-h urinary excretion of lometrexol was found to correlate linearly with the dose administered (r = 0.69; P < 0.01) even when these patients were omitted from the analysis, and a significant rank correlation was also evident (rₚ = 0.72; P < 0.001).

Pharmacokinetics of Lometrexol Administered without Folic Acid Supplementation. Lometrexol pharmacokinetics were studied in seven patients who received 45 or 60 mg/m² lometrexol without folic acid supplementation. A triexponential compartmental model was found to best describe plasma elimination of lometrexol in four of seven patients (Table 3) and a biexponential curve in the remainder. For most data sets a good curve fit was observed, the coefficient of determination (r²) being greater than 0.89 in every case (median, 0.99; range, 0.89–0.99). The t_{1/2}α and t_{1/2}β determined from these analyses were 17 ± 8 min and 169 ± 51 min, respectively, with a t_{1/2}γ (where measurable) of 2593 ± 1671 min. As was found with patients receiving folate acid, AUC and Cl_{TOT} calculated using model-dependent analysis were not significantly different from those calculated using model-independent analysis (P = 0.30 and P = 1.00). The lometrexol Vdᵦ in these patients was highly variable, i.e., 5.7–28.0 liters/m² and 8.5–31.8 liters/m² at 45 and 60 mg/m² lometrexol, respectively.

Plasma protein binding of lometrexol in patients treated without folate supplementation was virtually identical to that in patients receiving folate acid, with a value for the unbound lometrexol fraction of 22 ± 4% and 78 ± 4% for the protein bound fraction.
DISCUSSION

This study describes the first detailed examination of the plasma pharmacokinetics of lometrexol, a prototype antipurine antifolate, administered as an i.v. bolus to patients receiving folic acid supplementation. The plasma elimination of lometrexol was adequately described by a biexponential equation when data are collected up to 12 h after drug administration, although the inclusion of samples taken at later time points (i.e., 24 h and greater) would indicate that a triphasic model was a more appropriate description. The α, β, and γ phase half-lives determined for lometrexol were comparable to those measured for the classical dihydrofolate reductase inhibitors methotrexate (16) and edatrexate (10-ethyl-10-deaza-aminopterin; Ref. 17), but not with the thymidylate synthase inhibitor Tomudex, which differs in having a much longer τγ of 50–100 h (18). The data reported here agree with those of Young et al. (5) who performed a preliminary pharmacokinetic study of lometrexol in patients not receiving folic acid, using a particle concentration immunofluorescence assay, and identified biphasic and triphasic plasma pharmacokinetics. A tertiary elimination phase could potentially result from release of lometrexol from hepatic stores or enterohepatic cycling of the drug; two possibilities which have also been proposed to describe the t1/2γ of methotrexate (19, 20). The involvement of enterohepatic cycling has also been implicated in the disposition of dichloromethotrexate in humans (21) and CB3988 (C2-desamo-C2-methyl-N10-propargyl-2′-trifluoromethyl-5,8-dideazafolic acid) in the rat (22). The involvement of such mechanisms in the disposition of lometrexol are supported by whole-body autoradiographic studies using 14C-labeled lometrexol in mice maintained on a folate-deficient diet, which indicate that the liver is the primary organ for drug accumulation (23). This terminal elimination phase indicates persistence of lometrexol, which could be related to the cumulative toxicity encountered in early Phase I studies.

The AUC in individual patients studied after multiple courses of lometrexol was found to be minimally cumulative in three patients and noncumulative in five patients. Minimal accumulation of lometrexol on repeated treatment was also described in the earlier Phase I study of Young et al. (5). Similarly, in patients receiving repeated Tomudex treatment, no accumulation is evident (18). Lometrexol AUC was found to increase linearly with dose, an observation which is in agreement with data for other classical antifolates such as CB3717 (N7-propargyl-5,8-dideazafolic acid; Ref. 24), Tomudex (18), and edatrexate (17), but not for methotrexate for which it has been suggested that there is a nonlinear relationship between dose and AUC following bolus administration (25).

That there was a tendency for the lometrexol Vd∞ (and unbound Vd∞) to be larger in patients receiving higher doses of the drug may be indicative of concentration-dependent protein binding. Thus, protein binding of lometrexol may be a saturable phenomenon, with greater drug concentrations resulting in a larger unbound fraction. Unbound lometrexol would be subject to rapid cellular uptake and hence an apparently larger Vd∞. This possibility is supported by the observation that the fraction of lometrexol unbound is inversely related to serum albumin concentration (Fig. 4). A relationship between Vd∞ and dose has also been observed in pharmacokinetic studies with Tomudex in rats (26).

The Vd∞ range (4.7–15.8 liters/m²) from patients treated with 12–45 mg/m² lometrexol was, on average, smaller than that measured in patients who did not receive folic supplementation, but were treated with 45 and 60 mg/m² lometrexol (5.7–31.8 liters/m²), and that reported for patients treated in a previous study (14–32 liters/m²) with doses of 15–60 mg/m² (27). This observation may again reflect the possible concentration-dependent protein binding of lometrexol and the effect that higher lometrexol concentrations would therefore have on drug disposition.

Despite these findings, the in vitro protein-binding data did not indicate that concentration-dependent protein binding of lometrexol was statistically significant (P > 0.05) between the concentration ranges of 3.5 and 4.7 and 7.7 and 21.0 μg/ml, although a comparison of binding by a paired Student’s t test resulted in a P value of 0.11, which would indicate a trend toward reduced lometrexol binding at higher concentrations.

The magnitude of lometrexol protein binding (78%) was comparable to that of methotrexate (28, 29), but not with the thymidylate synthase inhibitors CB3717 and Tomudex which bind more extensively (97% and >90%; Refs. 24 and 26). Since lometrexol has a high affinity for membrane-bound FBP (30, 31), it should be noted that a component of the protein binding measured in this study may have involved binding to soluble FBP. Soluble FBP present in plasma is thought to function as a folate transport protein (32) and has a MI, 35,000–100,000 (33), which is above the threshold (30,000) used to determine protein binding.

Any condition influencing lometrexol protein binding, e.g., changes in protein conformation, hyperbilirubinemia, or displacement by concomitant drugs, could increase the cellular uptake and renal clearance of the drug. The inverse linear relationship between unbound lometrexol and the concentration of albumin, which comprises approximately 50% of plasma
proteins, also suggests that albumin concentration may be such a factor. As a determinant of lometrexol toxicity, however, plasma protein binding may be of lesser importance if drug dissociation is induced by high-affinity binding to membrane-bound FBP or the reduced folate carrier, proteins which facilitate the cellular uptake of lometrexol. This could also hold true for renal excretion, if renal tubular cells contain proteins that are involved in active secretion of lometrexol.

Renal excretion was found to be the major route of lometrexol elimination, with approximately 85% of the administered dose excreted as unchanged drug within 24 h after bolus i.v. injection, the majority of which (56%) occurred within the first 6 h. The percentage of the lometrexol dose excreted in urine was found to be comparable to that reported for methotrexate (70-94%; Ref. 34), but much greater than that for other antifolates in donation (22), while in comparison, the lipophilic nonpolyglutamatable dihydrofolate reductase inhibitor trimetrexate is found to undergo extensive systemic metabolism at the range of doses studied. Quinazoline antifolates are also poorly metabolized (22), while in comparison, the lipophilic nonpolyglutamatable dihydrofolate reductase inhibitor trimetrexate is found to undergo 95% biotransformation (38).

Folic acid supplementation reduces the toxicity of lometrexol, enabling the administration of up to four repeated courses of treatment (39). The mechanism(s) which underlies modulation of lometrexol toxicity remains to be identified, but the possibility exists that folic acid may increase the plasma clearance of lometrexol, such that the AUC is lower for a given dose. However, this would seem unlikely since comparison of plasma pharmacokinetic parameters for patients receiving 45 mg/m² lometrexol every 4 weeks, with or without folate supplementation, showed no marked differences. Unfortunately, samples were not available to study the urinary excretion and renal clearance of lometrexol in patients not receiving folic acid.

The disposition of lometrexol administered with a folate supplement is similar to that of methotrexate in terms of the kinetics of plasma elimination, extent of protein binding, and urinary excretion. Plasma elimination of lometrexol was not markedly different in patients who did not receive a folate supplement before and during lometrexol administration, and the improved tolerability of the drug when given with folate supplementation is therefore unlikely to be due to a pharmacokinetic interaction.

ACKNOWLEDGMENTS

We thank Drs. H. Schmidt (Lilly Research, Brussels, Belgium) and J. Walling (Lilly Research, Basingstoke, Surrey, United Kingdom) for supplying lometrexol and C10-desmethyl lometrexol, and for their support and encouragement. Thanks are also due to the research nurses, data managers, and medical staff in the Department of Medical Oncology, Newcastle General Hospital, who cared for the patients treated with lometrexol and obtained samples for the pharmacokinetic study. Finally, the help of Drs. C. Sessa, O. Pagani, and F. Cavalli in supplying plasma samples from patients treated without folate supplements is greatly appreciated.

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Lometrexol with Folic Acid Supplementation


Clinical pharmacokinetics of the antipurine antifolate (6R)-5,10-dideaza-5,6,7,8-tetrahydrofolic acid (Lometrexol) administered with an oral folic acid supplement.

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