

RESEARCH COMMUNICATION

Population Pharmacokinetics of Methotrexate in Indian Cancer Patients

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Abstract

Aims: A population pharmacokinetic model was developed to describe dose-response Relationships of methotrexate (MTX) in adults with breast cancer; this is done in order to explore interindividual variability in relationships with different pathophysiological variables. **Methods:** Forty-five patients receiving 122 courses of MTX (2-3 per patient) were included and data were analyzed using NONMEM software. A linear two-compartment model with linear elimination best described the data. The predictive performance was evaluated by comparing the predicted and observed concentrations and the population estimated parameters with the individual estimated parameters. **Results:** The population pharmacokinetic parameters CL, V₁, Q, V₂, K₁₂ and K₂₁ generated in NONMEM, using the FO method were 3.5 L/h, 1.25 L, 8.43 L/h, 6.45 L, 2.8, 6.74 and 1.30 h⁻¹ respectively. No covariate had significant effects on CL and VD. **Conclusions:** The results of this study combine relationships between the pharmacokinetic parameters of MTX and patient covariates that may be useful for dose adjustment, with a convenient sampling procedure that may aid in optimizing cancer patient care.

Keywords: Methrexate - population pharmacokinetics - patient covariates - India

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Introduction

Methotrexate (MTX) is an analog of aminopterin, a folinic acid antagonist, used as an anticancer agent. The therapeutic drug monitoring is essential for clinical management of patients receiving MTX. This is due to the wide inter and intra individual variabilities, as well as the well established relationship between MTX efficacy, toxicity and pharmacokinetics. Concerning its efficacy, the plasma maximal concentration (C_{max}) and the area under the time-concentration curve (AUC) are the major prognosis factors in histological response and disease free survival (Crews et al., 2004). In addition, a steady state of concentration greater than 16 μmol-l is associated with decrease risk of relapse (Evans et al., 1986). The systemic toxicity has been demonstrated to be directly related to MTX plasma concentrations, exposure time and the AUC (Ferreri et al., 2004). It also depends on to the dose adjustment and administration schedule of folinic acid rescue. This explains the importance of the strict monitoring of plasma concentrations during hospitalization until reaching a level below the threshold value of 0.05 μmol-l.

High dose intravenous methotrexate (HD-MTX) is an important component of many chemotherapeutic protocols (Moe and Holen, 2000) although the superiority

of high versus low dose MTX is still a matter of debate (Cohen, 2004). Several studies on MTX pharmacokinetics in children have been performed (Relling et al., 1994). Conventional compartmental or non compartmental approach (Rask et al., 1998) and population approach (Rousseau et al., 2002) have been used to compute individual pharmacokinetic parameters. In some studies, pharmacokinetic parameters were estimated by parametric and nonparametric methods using the software package USC*PACK (Aquerreta et al., 2000) or by using a Bayesian algorithm implemented in the software package ADAPT (Wall et al., 2000). The disposition of MTX has been well studied. It binds almost exclusively to the albumin fraction of human serum proteins. Renal secretion constitutes the major route of MTX elimination. MTX is filtered by the glomeruli and actively secreted by the proximal tubule. Overall, significant interindividual variability in the pharmacokinetic parameters of MTX has been observed (Bannwarth et al., 1994).

The objectives of our study were to develop a population pharmacokinetic model of low dose MTX (60 mg/m²) in patient with breast cancer, to study the effects of age, sex and other covariates on the serum level/control of cancer with MTX and on its population pharmacokinetic parameters like Clearance and Volume of distribution. This would enable the clinician to adjust the dosage schedule

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and to predict hospitalization duration so as to improve the patient health care provision.

Materials and Methods

Patients

Forty-five patients (2 men, 43 women) were studied. All patients were treated with MTX by the short infusion for at least 5 minutes. Patients were excluded if they were less than 18 years of age, and had hepatic disease or unstable, decompensated pulmonary, cardiovascular, gastrointestinal, endocrine, or renal disease. All prescribed concurrent medication was continued throughout the study, all of the included patients received concomitant medication like dexamethasone, ranitidine and ondansetran. Before entry into the study general health was assessed on the basis of medical history and physical examination, including temperature, heart rate, blood pressure, liver and renal function tests. Among the clinical and demographic parameters collected, only age, weight, height, sex were considered as clinically relevant. These parameters were used as potentially explanatory factors (covariables) of the pharmacokinetic interindividual variability.

The study protocol was in accordance with the legal requirements and was approved by the regional Ethics Committee. The patient group was selected from the cancer patients who visited cancer department in the Mahatma Gandhi Memorial Hospital (M.G.M.) located in southern India. Informed consent was taken from the patients and all the patients who were willing to participate in the study were taken after due permission from the Department of Cancer, M.G.M. Hospital. All the patients were enrolled in the Pop PK study and following information was collected from each one of the patient. Name, age, sex, bodyweight, type of cancer, family history, present treatment with starting date and dose, co medication, side effects, concomitant diseases (liver disorder / renal failure / CV disorders), work style, date and time of last dose taken and sample time.

Sample collection

Each patient received 60 mg/m² MTX by i.v infusion. After infusion, serial venous blood samples for drug assay were drawn in blood collection tubes by direct venipuncture or through an indwelling catheter inserted into an antecubital vein for repeated blood sampling. Approximately 5 ml blood was collected at three different time points after drug administration. Within 4hrs of collection, each sample was centrifuged at ambient temperature for 5 min at 3000 g. The resultant serum was separated, transferred into prelabelled polypropylene tubes, and promptly frozen at - 80°C until they were assayed by High Performance Liquid Chromatography [HPLC].

Analytical method

MTX concentrations in serum samples were assayed by a HPLC-UV method. The limit of quantification (LOQ) was 5µM. Quality control assessments were done during the whole study period. HPLC assay is based on published method (Abdolhosein et al., 2003). Briefly, to each

100µL of patient's or standard sample, 100µL of stock solutions of internal standard (Para aminoacetophenone) 5µg/mL was added. After complete mixing of samples with internal standard, 40 µL of trichloroacetic acid (2 M in ethanol) was added and vortex mixed for 2 minutes, then centrifuged at 3000 rpm for 15 minutes. 10 or 20µL aliquots of the supernatant was directly injected into the chromatography column. Each sample was analyzed in duplicate.

Pharmacostatistical analysis

The statistical pharmacokinetic program, non linear mixed-effect model was performed using the software NONMEM VI 1.1 Version, was used to determine the population pharmacokinetic parameters of MTX. The individual parameters were determined according to a two-compartment model. The first order [FO] algorithm was used, retaining interaction between interindividual random effects and the residual error term. Selection of structural models was based on the fit of the model to the data, consistent with reliable parameter estimation and significant change in objective function. Models incorporated interindividual variability in all parameters, as well as interindividual variability between patients. The influence of patient covariates on clearance and volume of distribution parameters were investigated. Decisions on the fit of models to the data and the inclusion of covariates were based on objective function values, plots of parameters versus covariates and significance of coefficients (Batey et al., 2002).

Estimation of population parameters

Covariates tested in the NONMEM evaluation included age, height, body weight and sex. The data set used to develop the population pharmacokinetic model was analyzed for the presence of obvious outliers which were deleted. The structural model was developed using the following pharmacokinetic models: two-compartment first-order elimination (ADVAN 3 TRANS 3).

For the structural model the covariates evaluated were age, weight, height and dose. The allometric scaling transformations of weight and average weight were also assessed. A change in objective function of at least 3.8 (P < 0.05 with one degree of freedom) was required for statistical significance at the initial covariate screening stage. Finally, accepted covariates were added to the model and the population pharmacokinetic parameters were estimated. To demonstrate that retained covariates contributed to an improvement of the fit of the population pharmacokinetic model, each covariate was deleted sequentially from the proposed final model (backward elimination) in order to confirm statistical significance. If the objective function did not vary significantly, the relationship between the covariate and the pharmacokinetic parameter was ignored (Sheiner et al., 1977).

Results

The population data base consisted of 122 MTX concentrations obtained from 45 patients. Patient demographic data was shown in Table 1. The

Table 1. Range and Mean (SD) Values for Patients Given Methotrexate

	Range	Mean (SD)
Age (years)	35 –76	51.11 (10.5)
Dose (mg/kg/day)	50 – 60	52.22 (4.2)
Serum level ($\mu\text{g/ml}$)	1.01 – 46.4	6.4 (8.5)
Serum level/dose ratio	0.02 – 0.7	0.125 (0.16)
Body weight (kg)	27– 79	49.1 (11.5)
Sampling time (hrs)	0.08 – 3.3	0.53 (81)
(time lapsed after last dose)		

Table 2. Details of Population Models used for MTX using FO Algorithm

Model	OFV	Population estimate(%SE)	Between subject variability(%SE)
Base model	383.37		
CL(L/hr)		3.5 (7.1%)	0.003 (5%)
V1(L)		1.25 (18.6%)	0.0003 (1%)
Q(L/h)		8.43	0.207 (45%)
V2(L)		6.45	0.086(29%)
K (h-1)		2.8	
K12		6.74	
K21		1.30	
Proportional error		20% (0.042)	

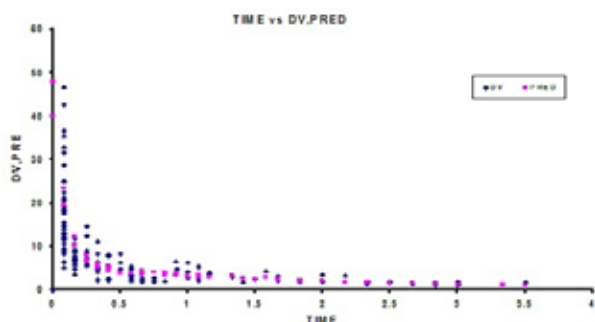


Figure 1. Serum Concentration Time(hrs) Profiles of MTX DV-Observed Concentrations; PRED-Predicted serum MTX concentrations

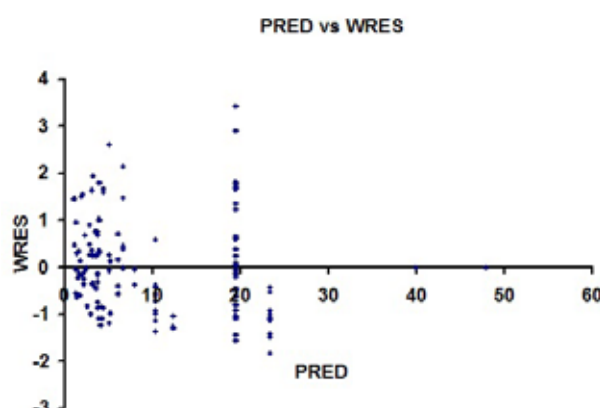


Figure 2. Scatter Plot of Predicted versus Weighted Residuals [Base model]

pharmacokinetic parameters of the patient population computed using NONMEM. Figure 1 shows that the Time Vs observed and predicted concentrations of MTX in all patients. A two-compartment model was fitted to the data, with interindividual effect on all parameters using FO method (Table 2). FOCE algorithm was not fit for the

TIME vs WRES

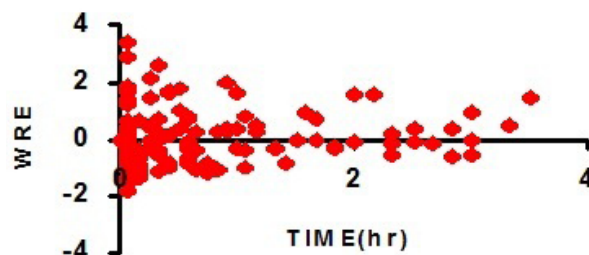


Figure 3. Scatter Plot of Time versus Weighted Residuals [Base model]

PRED vs DV

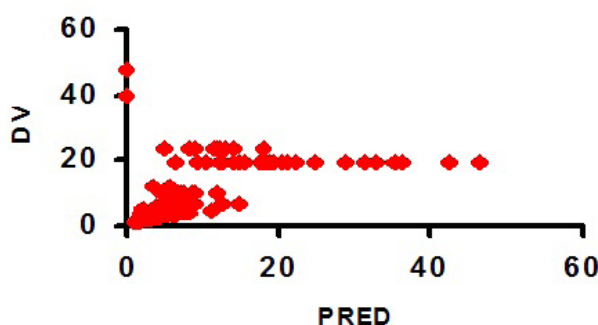


Figure 4. Scatter Plot of Observed Serum Concentrations versus Predicted Concentrations

data. A proportional error model best described the pattern of residual error and the raw data were log-transformed to minimize the dependence of mean parameters on estimation of the variance function. The strength of the relationships between the various covariates like total body weight, height, age or dose was shown by hypothesis testing of full-reduced models during covariate screening.

A residual is the difference between an observation and its prediction. The prediction in this case is the population prediction i.e., the prediction for the typical individual having the given values for all the concomitant variables. With population data, weighted residuals are often more informative than (plain) residuals. The weighted residuals for an individual are formed by transforming the individual's residuals so that under the population model, and assuming the true values of the population parameters are given by the estimates of those parameters, all weighted residuals have unit variance and are uncorrelated.

None of the available covariates contributed significantly to the variability in CL or V. The estimates of CL, V1, Q, V2, K, K12 and K21 generated in NONMEM, using FO method were 3.5 L/h, 1.25 L, 8.43 L/h, 6.45 L, 2.8, 6.74 and 1.30 h⁻¹ respectively. Serum concentration time profiles; Time Vs observed and predicted concentrations of MTX in all patients were shown in Figure.1. The plots of the observed serum MTX concentrations and weighed residuals against predicted serum MTX concentrations for the basic PK and final models were shown in Figures 2-4.

To test which particular parameter values rendered the data most probable, objective functions were compared

between successive models. To build full regression model, a difference in objective function values was required to indicate that the model with the lowest objective function was probably better than another (Otero et al., 1996).

The final model was determined from the full model by removing each covariate one by one, using a more restrictive criterion. In addition to minimum value of objective function values, residual plots, standard error and correlation matrix of the parameter estimates and size of the interindividual variance in CL and V are also considered in choosing the models.

Discussion

The principal aim of Population pharmacokinetic analysis is to account for the inherent kinetic variability in a population of patients in terms of a number of readily identifiable factors (Early Breast Cancer Trialists' Collaborative Group, 1992). A better understanding of the intra- and interindividual variabilities associated with the pharmacokinetic and pharmacodynamic behavior of therapeutic agents can lead to a more efficacious and safer drug use. These include physiologic, pathologic, and treatment design rational dosage guidelines that should result in therapeutic concentrations, based on sound quantitative analyses rather than on purely empiric considerations, in the majority of patients. The main application of population models is to establish dosage regimens. Apart from this, it is possible also to estimate the variability of the concentrations achieved, which, for any given dosage regimen, should permit calculation of the proportion of patients at risk of attaining toxic or ineffective concentrations.

Adverse events associated with low-dose MTX in breast cancer have been reported (Mc Kendry and Dale, 1993), however, their incidence is more important in elderly patients or in patients with renal insufficiency, hypoalbuminaemia, or intercurrent illnesses, or when concomitant treatments, potentially nephrotoxic, are used (Iliadis et al., 1985). A great interindividual variability in MTX pharmacokinetic parameters and a great variability in individual responses to MTX was observed.

The present study is unique that it is the first population pharmacokinetics study of anti cancer drug MTX done in INDI A using a Non Linear Mixed Effects Modeling. Our study population was representative of the population of India.

We developed a population pharmacokinetic model for adults with breast cancer and evaluate the influence of covariates on pharmacokinetics. A high inter and intra individual pharmacokinetic variability for high dose MTX was observed (Monjanel-Mouterde et al., 2002). The two compartment model revealed to be the best model describing the pharmacokinetics of MTX (Joerger et al., 2006).

M.A. Batey et al (2002) reported that that the MTX clearance has been shown to be related to the GFR only at low concentrations (less than 0.4 mM). Tubular secretion and reabsorption are also important and the latter is saturable at the concentrations attained after a

short infusion. The disposition of methotrexate could be characterized by two or three compartmental model with first order absorption and elimination. A two compartmental model with first order elimination was best fitted to the present data.

In our study the volume of distribution is very low when compare with past reports unlike other studies here there is no covariate affect the CL and V in breast cancer patients.

MTX followed two compartment models, which is already observed in other studies.

The results of the present study indicate that optimization of MTX dosing may provide further improvement in chemotherapy of cancer, although the potential benefit, in terms of uniformity of plasma concentration time profiles may be limited by interindividual variability. This might be a step forward in the effort to ensure a more optimal and individualized MTX therapy.

The population pharmacokinetic parameter estimation of methotrexate in breast cancer patients was well described by this investigation. Population PK models were developed and influences of different covariates were studied. The results of the present study show that the CL and V values of MTX were found to be 3.5 L/h and 1.25L respectively which are very low. We also confirmed that the individualization strategies based on pharmacokinetics have the potential to improve the risk/benefit relationship of MTX. In clinical practice, this approach can be a valuable tool for prediction of individual MTX parameters after i.v infusion.

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