POPULATION PHARMACOKINETICS OF 5-FLOURO URACIL IN INDIAN CANCER PATIENT POPULATION

NAGULU MALOTHU*[1], UDAY KIRAN VELDANDI[1], NARSIMHA REDDY YELLU[1],NALINI YADALA[2], RAMA KRISHNA DEVARAKONDA[1]

[1]Drug Metabolism and Clinical Pharmacokinetics Division, University College of Pharmaceutical Sciences,
[2]Kakatiya medical college, Kakatiya University, Warangal, AP – 506 009, India. E mail: nagoncology@gmail.com

ABSTRACT

The aim of the present study was to describe the population pharmacokinetics of 5-flourouracil in Indian cancer patient population. The covariates evaluated were total body weight, height, age, dose and gender. A total of 85 steady state serum concentrations were collected from 44 patients and analyzed. 5-FU pharmacokinetics is followed by one compartment first order elimination. Additive model is best described the pattern of residual error using both FO and FOCE method. NONMEM was performed to investigate the effect of patient covariates on pharmacokinetics and to investigate the relative magnitude of inter individual variability. No covariate is influencing the CL and VD of 5-FU.The final model estimates of CL/F and V/F estimated by FO method were 74.5/h and 11.8 L respectively and by FOCE method are 72.3 L/h and 12 L respectively.

Key words: Cancer, 5-Fluro uracil, Population pharmacokinetics.

INTRODUCTION

The important application of population pharmacokinetics (PK) in oncology is based on the assumption that clear pharmacodynamic relationships exist between plasma drug concentrations and clinical effect. The latter should include antitumour effects but, more commonly, toxicity has been used as a pharmacodynamic endpoint. A further assumption is that accounting for PK variability will result in optimal dose individualisation.Such an approach has been applied for carboplatin [1], docetaxel [2] and epirubicin [3] Methotrexate [4].

5-Flourouracil is an anti metabolite and pyrimidine analogue which is widely used for the treatment of cancer in adults principally as a thymidylate synthase inhibitor [5].

However, it is difficult to establish suitable dosage regimens for this drug because of the lack of a good relation between the dose and the desired effect, variations in its pharmacokinetic characteristics [6]. The influence of genetic differences, age, sex, variable absorption rates, auto induction, disease states, and comedication may cause significant changes in blood levels of 5-Fu and its metabolites [7]. Thus knowledge of 5-Fu pharmacokinetics in different population groups is essential to ensure safety and efficacy. Dosage adjustments based on individual pharmacokinetic parameters are of considerable importance for the effective and safe use of drugs.

Obtaining adequate number of blood samples from each individual to characterize the concentration-time profile is difficult in practical scenario. To obviate the need for multiple blood sampling, a population approach can be used.

Population pharmacokinetics seeks to discover which measurable pathophysiological factors cause changes in the dose-concentration relationship and to what degree so that appropriate dosage can be recommended. NONMEM (Nonlinear Mixed Effects Model) software is used for the population analysis approach [8].

The chief advantage of the population approach is that useful information can be extracted from sparse data obtained during routine clinical care in order to estimate mean kinetic parameters together with interindividual and residual variability. NONMEM allows the estimation of population average values of pharmacokinetic parameters, such as volume of distribution (V) and clearance (CL) together with estimates of the interindividual variability in the pharmacokinetic parameters, which can be related to the modifying influence of demographic and clinical factors (covariates).

Extensive work on pop pk of anti cancer drugs CMF (Methotrexate,5-FU and Cyclophosphamide) has been reported in most Caucasian, Sweden and German populations but the data is scarce on Indian cancer patient population .The population pharmacokinetic parameters of 5-FU in these populations were reported through different investigations [4,7,9]. There are no reports on population pharmacokinetic parameters of any of these drugs in Indian population. Hence we took up the estimation of population pharmacokinetic parameters in Indian patient population using Non Linear Mixed Effects Modeling, NONMEM. The purpose of the current study, the 5-FU chemotherapy of breast cancer, offers an opportunity to explore these issues in a clinically-relevant setting.

This study was undertaken in Indian patients of cancer, to investigate

1. Estimation of population pharmacokinetic parameters of 5-Fu, an anti cancer drug.

2. The effects of age, sex and other covariates on the serum level/control of cancer with 5-Fu and on its population pharmacokinetic parameters like Clearance and Volume of distribution.

PATIENTS AND METHODS

The patient group was selected from the cancer patients who visited cancer department in the Mahatma Gandhi Memorial Hospital (M.G.M.). 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. Institutional ethics committee approval was taken before starting the study. All the patients were enrolled in the Pop PK study and following information were collected from each one of the patient Name, age, sex, bodyweight, type of cancer, biochemical and electrophysiological investigations (Serum electrolytes, Blood Urea Nitrogen, Serum creatinine,). 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. In this study, 44 cancer patients were selected. All the patients were on 5-FU, Methotrexate (MTX) and Cyclophosphamide (CPH) multi therapy. Drugs for administration were prepared according to the manufacturer's instructions and administered as short i.v. infusions (1–5 min).The exact start and stop times of each drug administration were carefully recorded. Only adult patients were included in this study. Blood samples [5ml] were collected three samples at different time   points, centrifuged and the serum samples were labeled and stored at – 80⁰ C until analysis was done.

Drug Analysis

5-Fu serum samples were analyzed using a High performance liquid chromatography (HPLC) assay based on published method [10]. Briefly, different concentrations of 5-FU were first added to 50μl of thawed serum samples which were vortex-mixed for 2 min. Then, 50μl of 10 μg/ml internal standard was added. A solution of 1ml of ethyl acetate, as an extracted solvent, was added to the serum samples. Samples were vortex-mixed for 7 min and then centrifuged (4000 g, 10 min). The supernatant was collected and the organic extraction process was repeated collecting organic supernatant into the same glass tube. Samples were evaporated by heating on water bath and reconstituted in 200μl of water, vertex mixed. 10 or 20μL aliquots of the supernatant was directly injected into the chromatography column.

Pharmacostatistical analysis

The population pharmacokinetic analyses were performed using NONMEM (version 5) in conjunction with a Fortran Power station compiler. The first order estimation method was used and the estimates from the final model were confirmed using the first-order conditional estimation method for interaction. Data files were constructed using Excel for Windows 98. The NONMEM output was processed further using Excel. All the demographic data (age, sex, weight, height), concentration obtained at different time intervals, dosing pattern and schedule were used in the preparation of the data file. 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: one-compartment first- order elimination (ADVAN 1 TRANS 1).

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. Each covariate was added to the basic pharmacokinetic model and the objective function value was noted. An analysis was then performed by a forward, stepwise technique where each covariate which individually caused a decrease in objective function value was added cumulatively to the model. This process was continued until no further reduction in the objective function value resulted. Finally, a backwards elimination step was performed by setting the coefficient of each covariate, in turn, to zero and nothing the change in the objective function value.

RESULTS

The data comprised of two to three observations (serum samples at 3 different time points after infusion of drug (post dose)) from each of 44 subjects. In total, 85 observations were obtained. There was a wide distribution of height, weight and sex. Total 42 female and 2 male were included in the study. The concentration-time course of 5-Fu was described by using a one-compartment model with first order elimination. A one-compartment with first-order elimination (subroutine ADVAN 1) was considered the most appropriate PK model. In the process of model building index plots were used to identify gross errors.

An additive model was used to describe the inter-individual variability best. Though initially done with exponential (logarithmic) error model, satisfactory result was not obtained. The strength of the relationships between the various covariates like total body weight (TBW), 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. Total 44 patients recruited to the study, data was available for 85 samples. The patient characteristics are given in Table 1.

Table 1: Range and mean (±SD) values for patients under 5-Flourouracil treatment

Patient characteristics	5-Fu patients	
	Range	Mean (±SD)
Age (years)	35 – 76	51.6 (±10.3)
Dose (mg/kg/day)	500 – 900	641.9(±140.5)
Serum level (μg/ml)	0.08– 0.4	0.12 (±0.18)
Serum level/dose ratio	0.003 – 0.09	0.02 (±0.02)
Body weight (kgs)	30– 79	49.1 (±11.5)
Height (cms)	125 - 179	147.5 (±10.2)
Sampling time(hrs)	0.08-3.3	0.12 (±0.18)

Table 2: Details of population models used for 5-FU

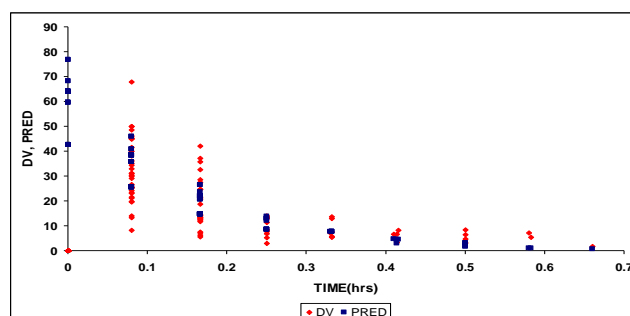| FOCE MODEL			
Model	OFV	Population estimate (%SE)	Between subject variability (%SE)
Base model	460.677		
CL(L/hr)		72.4 (6.9%)	0.24 (68.5%)
V(L)		12 (12.4%)	0.44 (60.2%)
Residual variability			
Additive error		6.76μg/ml(25.9)	
FO MODEL			
Model	OFV	Population estimate (%SE)	Between subject variability(%SE)
Base model	462.121		
CL(L/hr)		74.5 (6.2%)	0.23 (113%)
V(L)		11.8 (15.5%)	0.40 (55.7%)
Residual variability			
Additive error	7.21μg/ml (52.1)		

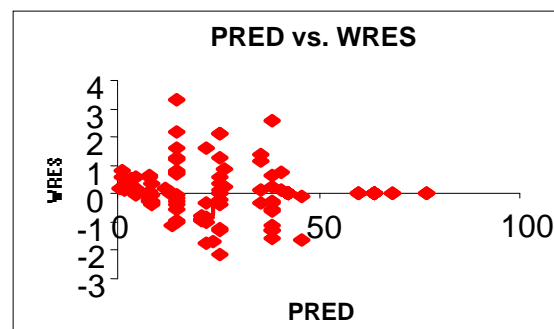**Figure 1:** Serum concentration time profiles of 5-FU in all patients

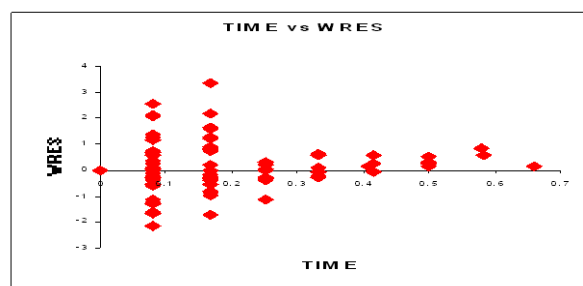**Figure 2:** Scatter plot of predicted versus weighted residuals [Base model]

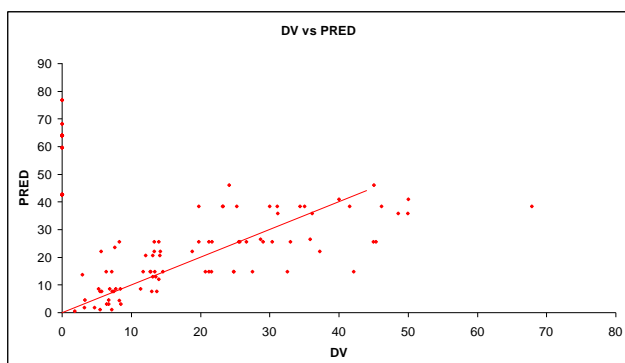**Figure 3:** Scatter plot of Time versus weighted residuals [Base model]

Figure 4: Scatter plot of Observed serum 5-FU concentrations versus predicted concentrations

Serum concentration time profiles; Time Vs observed and predicted concentrations of 5-FU in all patients are shown in Figure 1.

The pharmacokinetics of 5-FU could parsimoniously be described by a one-compartment model with interindividual and interoccasional random effects on clearance .An additive model is best described the pattern of residual error using both FO and FOCE methods (Table 2). No evidence of a second compartment or of nonlinear pharmacokinetics could be discerned in these data from a limited dose range.

The plots of the observed serum 5-FU concentrations and weighed residuals against predicted serum 5-FU concentrations for the basic PK and final models were shown in Figures 2 – 4. None of the available covariates contributed significantly to the variability in CL or V. The estimates of CL and V generated in NONMEM using the final model were 72.3 L/hr and 12 L/hr respectively.

In the basic regression model (CL = θ_1), additional parameters were incorporated in a stepwise fashion to develop intermediate and the full models. TBW, the dose of the 5-FU, and age were included, as continuous covariates.

Discrete covariate like sex was assigned a value of zero for females and one for males. A constant CV model was used to describe the error (η_{CL}) between individual (CL_{ij}) and the population mean (CL^1). An additive model was used to describe the residual error (ϵ_{ij}), between the observed (C_{ij}) and the predicted (C^1_{ij}) concentrations. η_{CL} and ϵ_{ij} were assumed to be independent and normally distributed with mean zero and variances ω^2 and σ^2, respectively.

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 [11].

The final model was determined from the full model by removing each factor 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 inter individual variance in CL and V are also considered in choosing the models.

DISCUSSION

The concept of individualization of drug doses in cancer chemotherapy has been applied for a number of agents. The most common application is the dosing of carboplatin to a target AUC, using a measure of GFR to calculate clearance [12]. The utility of therapeutic monitoring of etoposide during continuous infusion has also been demonstrated [13], and modulation of the MTX dose to achieve a target plasma concentration improves outcome in patients with leukaemia [14]. Pop pk of adjuvant treatment of early stage breast cancer with CMF [4] and Patients receive repeated courses of chemotherapy, thus allowing for adaptive dosing following course 1, and a dose–response relationship in terms of survival and time to relapse has been established [15].

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 [16]. A better understanding of the intra- and inter individual variability 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.

Estimation of pharmacokinetic parameters in the target population rather than implementation of parameters derived from normal healthy volunteers or western populations to improve individual estimation is highly desirable and will help to improve our population kinetic profile.

The present study is unique that it is the first population pharmacokinetics study of anti cancer drug 5-FU done in INDIA using a Non Linear Mixed Effects Modeling. Our study population was representative of the population of India. Serum concentrations are still being used clinically either to assess the dose titration or to monitor potential toxicity. Several factors are known to influence the relationship between dose and steady-state level of 5-FU including age, gender, genetic differences, weight, variability in absorption, dose-dependent auto induction, disease states and concomitant medication [7].

Previously, one study of the pharmacokinetics of CMF, specifically as used in the adjuvant treatment of breast cancer, has been reported. In a cohort of 23 women undergoing adjuvant chemotherapy with the CMF regimen, the interpatient variability in the AUC for each of the component drugs of the regimen was found to be 3- to 4-fold .Interoccasional variation in the pharmacology of CMF was less than 50% in the majority of patients, i.e. substantially less than the intersubject variability [17].

M.A. Batey et al[4] reported that the estimates of interoccasional and interindividual variability for 5-FU were small (14%) and approximately equal. The validity of these estimates may be limited due to the assumption of a linear single-compartment model and the high residual error (31%).In this relatively small initial patient population, no covariate was found to significantly influence the pharmacokinetics of 5-FU.

In our study the estimates of interoccasional and interindividual variability for 5-FU were small and approximately equal. The validity of these estimates may be limited due to the assumption of a linear single-compartment model and the high residual error. When data from studies using a longer duration of infusion have been analysed, several important covariates were identified. These included time of day, peripheral mononuclear cell dihydropyrimidine dehydrogenase and elapsed infusion time, none of which were available or applicable to the current study. In addition, clearance appeared to decrease when a raised alkaline phosphatase was reported and with increasing age [18].The effect of alkaline phosphatase was not observed in the current study and no covariate was found to significantly influence the clearance of 5-FU.

Age appeared to influence clearance and patients had a higher clearance. This unexpected finding may be spurious, or may reflect some other unrecorded covariate. In a previous population analysis of 5-FU PK, a saturable clearance model was used [19], although the estimated Km was high (27 mg/ml) relative to the concentrations observed in our data set.

Adaptive dosing of 5-FU has been attempted, but only when administered as a longer infusion. For example, a study of the relationship between AUC and toxicity in 89 patients with head and neck cancer [20], led to the development of a strategy for dose reduction in patients whose AUC following initial therapy exceeded

a certain threshold. A reduction in the incidence of toxicity was associated with fewer dose delays and a higher response rate. The improvement in therapeutic index may be related to the proportion of early-stage patients in the two arms of the study. Applying such an approach based on the analysis performed in the current study would require a larger study with more pharmacodynamic information in order to identify target plasma concentrations.

The results of the present study indicate that optimization of 5-FU 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 interoccasional variability. This might be a step forward in the effort to ensure a more optimal and individualized 5-FU therapy.

CONCLUSIONS

Using NONMEM software population pharmacokinetic parameter estimation was performed. PK models were developed and influences of different covariates were studied.

Using the above model the CL and V values of 5-Fu were found to be 72.3 L/h/kg and 12 L/kg respectively.

Strict adherence to the therapeutic range is justified during 5-Fu treatment and clinical control of patients needs not be attempted. There was no covariate that affects the modeling 5-FU population pharmacokinetics in adults. No change in CL and V was observed with any covariate.

REFERENCES

1. Chatelu t E, Canal P, Brunner V, et al.Predictio n of carboplatin clearance from standard morphological and biological patient characteristics. J Natl Cancer Inst 1995, 87, 573–580.
2. Bruno R, Vivier N, Vergniol JC, Dephillips SL, Montay G, Sheiner LB.A population pharmacokinetic model for docetaxel (Taxotere1)-model-building and validation. J Pharmacokin Biopharm 1996, 24, 153–172.
3. Wade JR, Kelman AW, Kerr DJ, Robert J, Whiting B. Variability in the pharmacokinetics of epirubucin—a population analysis. Cancer Chemother Pharmacol 1992, 29, 391–395.
4. Batey MA,Wright JG, Azzabi A, Newell DR,Lind NJ,Calvert AH ,Boddy AV.Population pharmacokinetics of adjuvant cyclophosphamide ,methotrexate and 5-flurouracil(CMF).European Journal of Cancer 2002;38:1081-1089.
5. Grace K. D and Alex A. A. Principles of Chemotherapy.Oncology 2006;Section One:14-40
6. Milano G, Etienne M, Renee N.Relationship between fluorouracil systemic exposure and tumor response and patient survival. J Clin Oncol 1994, 12, 1291–1295.
7. Sandstrom M, Lindamn H,Nygren P,Johansson M,Bergh J,karlson MO.Population analysis of the pharmacokinetics and the hematolohical toxicity fo the fluorouracil-epirubicin-cyclophosphamide regimen in breast cancer patients.Cnacer Chemother Pharmacolo 2006;58:143-156.
8. Aarons L. "Population Pharmacokinetics: Theory and Practice." Br J Clin Pharmacol 1991; 32: 669 - 670.
9. Wilde Stefan, Jetter Alexander, Rietbrock Stephan, Kasel Dirk, Engert Andreas, Josting Andreas, Klimm Beate, Hempel Georg, Reif, Stefanie, Jaehde Ulrich, Merkel Ute, Busse Dagmar, Schwab Matthias, Diehl Volker; Fuhr Uwe. Population Pharmacokinetics of the BEACOPP Polychemotherapy Regimen in Hodgkin's Lymphoma and its Effect on Myelotoxicity .Clinical Pharmacokinetics, 2007 ,:46:319-333(15).
10. Alsarra Ibrahim A,Alarifi Mohammed N. Validated liquid chromatographic determination of 5-fluorouracil in human plasma.Journal of chromatography 2004; 804:435-439.
11. Sheiner LB,Rosender B,Marathe VV.Estimation of population characteristics of pharmacokinetic parameters from routine clinical data .Journal of Pharmacokinet Biopharm 1977;5:445-79.
12. Calvert AH, Newell DR, Gumbrell LA.Carboplatin dosage: prospective evaluation of a simple formula based on renal function. Clin Oncol 1989, 7, 1748–1756.
13. Joel SP, Ellis P, O'Byrne K .Therapeutic tic monitoring of continuous infusion etoposide in small-cell lung cancer. Clin Oncol 1996, 14, 1903–1912.
14. Evans WE, Relling MV, Rodman JH, Crom WR, Boyett JM, Pui CH.Conventio nal compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. New Engl J Med 1998, 338, 499–505.
15. Bonadon na G, Valagussa P, Moliterni A, Zambetti M, Brambilla C.Adjuvant cyclophosphamide, methotrexate and fluorouracil in node-positive breast cancer. New Eng J Med 1995, 332, 901–906.
16. Otero MJ, Buelga D. Santos ,Vazquez M. A., Barrueco M. Dominguez-Gil A. Application of population pharmacokinetics to the optimization of theophylline therapy.Journal of Clinical Pharmacy & Therapeutics 1996; 21(2):113-125.
17. Moore MJ, Ehrlichman C, Thiessen JJ.Variability in the pharmacokinetics of cyclophosphamide, methotrexate and 5-fluorouracil in women receiving adjuvant treatment for breast cancer. Cancer Chemother Pharmacol 1994, 33, 472–476.
18. Tenet C, Erdociain E, Guimbaud R.Dose and time dependencies of 5-fluorouracil pharmacokinetics. Clin Pharmacol Ther 2000, 68, 270–279.
19. Sandstrom M, Freijs A, Larsson R.Lack of relationship between systemic exposure for the component drugs of the fluorouracil, epirubicin, and 4-hydroxycyclophosphamide regimen in breast cancer patients. J Clin Oncol 1996, 14, 1581–1588.
20. Santini J, Milano G, Thyss A.5-FU therapeutic monitoring with dose adjustment leads to an improved therapeutic index in head and neck cancer. Br J Cancer 1989, 59, 287–29.