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Variability in the pharmacokinetics of cyclophosphamide, methotrexate and 5-fluorouracil in women receiving adjuvant treatment for breast cancer

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Abstract. A total of 23 women with stage II breast cancer receiving adjuvant cyclophosphamide, methotrexate and 5-fluorouracil had detailed pharmacokinetic monitoring performed on the first and third courses of therapy. The area under the concentration time curve (AUC) of each of these three drugs varied by a factor of 3–4 among patients. No systematic change in pharmacokinetics between the first and third courses was seen for cyclophosphamide, methotrexate or 5-fluorouracil, and the mean AUC for each of the three drugs did not change. However, significant intrapatient variability in drug pharmacokinetics was observed for all three drugs such that the AUC, clearance and half-life in an individual on the third course could not be reliably predicted from data generated on the first course. On the basis of these results, cyclophosphamide, methotrexate, and 5-fluorouracil pharmacokinetic data from one treatment would not be useful information from which the doses for subsequent courses could be determined.

Introduction

The use of adjuvant chemotherapy with cyclophosphamide, methotrexate and 5-fluorouracil (CMF) has been demonstrated to reduce the risk of disease relapse after surgery for some women with breast cancer [2]. The importance of the given dose or "dose intensity" of these cytotoxic drugs when used as adjuvant therapy for breast cancer is an area of active debate [10]. Bonadonna and Valagussa [1] reported that women who received their full planned dose of CMF chemotherapy had a lower relapse rate than those who received less than 85% of the planned dose. A retrospective review of a number of adjuvant studies has demonstrated a relationship between the 3-year relapse-free survival and the planned dose intensity of CMF [11]. Prospective randomized studies to test the importance of dose intensity in women with stage II breast cancer are currently in progress in North America and Europe.

Plasma concentration profiles would be a more accurate measure of the systemic exposure to these cytotoxic drugs than the dose given [16]. Previous studies in cancer patients have shown considerable interindividual variability in plasma concentration profiles for all three drugs [5, 13, 15, 17]. Cyclophosphamide (CP), methotrexate (Mtx) and 5-fluorouracil (FUra) are currently given in a dose calculated on the basis of body surface area. Variability in pharmacokinetics and pharmacodynamics is considered only to the degree that patients experiencing moderate or severe toxicity have dose reductions made for subsequent courses of therapy. It is less common to escalate drug doses in patients experiencing minimal toxicity.

In this study a detailed analysis of the plasma concentration profiles of CP, Mtx and FUra was performed on the first and third courses of treatment in women with breast cancer receiving adjuvant chemotherapy. The study objectives were to determine the degree of inter- and intraindividual variability in plasma concentration profiles, to identify patient factors that might account for any variability seen and to determine whether the disposition of CP, Mtx or FUra would change in a predictable fashion with repeated administration. This study was the first step in a strategy to examine the feasibility of drug concentration monitoring to improve the therapeutic index of this treatment.

Patients and methods

Treatment. A total of 23 women with carcinoma of the breast undergoing adjuvant chemotherapy with CP, Mtx and FUra (600, 40 and 600 mg/m^2, respectively, given intravenously) were studied on their

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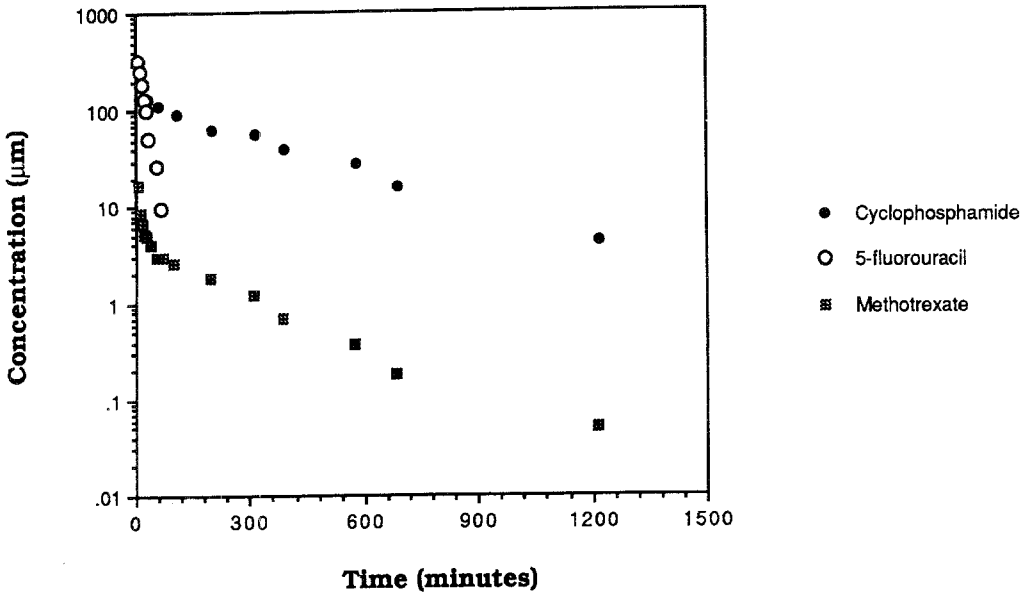

Fig. 1. Concentration-time profile obtained for CP, Mtx and FUra in a patient on the first course of therapy

first and third courses of therapy. All drugs were given by bolus injection in the sequence CP, Mtx, FUra. A standard anti-emetic regimen of 10 mg dexamethasone given intravenously and 10 mg prochlorperazine given orally was initiated prior to treatment. Cycles were repeated every 21 days if blood counts had returned to normal. All women had plasma creatinine levels of <120 μM (1.4 mg/dl), plasma bilirubin values of <20 μM (1.2 mg/dl), AST and ALT values within normal limits, a white blood count of $>4.0\times10^9$/l and a platelet count of $>150{,}000\times10^9$/l. None was on concurrent medications known to affect the metabolism of CP, Mtx or FUra (i.e. phenobarbital, acetylsalicylic acid).

Sampling. Blood was collected from an indwelling heparin-loc catheter placed in the arm opposite to that used for the administration of chemotherapy. Samples were taken prior to chemotherapy and at 5, 10, 15, 20, 30, 45, 60 and 90 min and 2, 4, 6, 12, 18 and 24 h after the start of the injection of CP. Time values for Mtx and FUra were adjusted to account for their precise time of injection. Plasma was separated and frozen at –20° C until analysis. CP and FUra were measured using previously described high-performance liquid chromatographic (HPLC) methods [3, 9], and Mtx was measured using fluorescence polarization immunoassay on the Abbott TDX. The coefficients of variation for Mtx, FUra and CP were $<10\%$ as assessed by serum pools spiked with drug, measured during the collection of each batch of specimens. Detection limits for the drugs were just below the lowest standards, at 0.05 μmol/l, 0.5 μmol/l and 0.5 mg/l for Mtx, FUra and CP, respectively.

Pharmacokinetic and statistical analysis. The drug concentration versus time data were analyzed via model-dependent and -independent analyses. For the model-dependent analysis, the data were computer-fitted to one or more compartmental models by means of a modified non-linear least-squares program [4, 8]. The data were weighted by the reciprocal square of the predicted values. Assignment of the model of choice was based on that leading to a statistically significant decrease in the sum of squares through the progressive addition of exponentials [8]. For the non-compartmental analysis, areas under the concentration-time curve (AUC) were determined by the trapezoidal method. The mean residence time (MRT) was determined by taking the ratio of the area under the moment curve to the AUC. Total body clearances were calculated as dose/AUC. Significance tests of the differences between courses 1 and 3 were performed using a paired *t*-test. Differences were considered significant if the probability (two-tailed) was less than 0.05.

Table 1. Patients' characteristics

Age	47	(32–66) years
Height	160.5	(147–171) cm
Weight	66.7	(52.2–89.3) kg
Body surface area	1.69	(1.48–1.90) m^2
Plasma creatinine	72.0	(41–103) μmol/l
Plasma bilirubin	9.2	(3–18) μmol/l
Number of positive axillary nodes	4.4	(1–19)

[a] Results expressed as mean values (range), $n = 23$

The associations between pharmacokinetic parameters were examined by calculating linear correlation coefficients between paired parameters. The significance of the correlations was determined using a two-tailed *t*-test. The association of pharmacokinetic parameters and plasma creatinine and creatinine clearance was determined in a similar manner. For paired comparisons between the first and third courses of chemotherapy, the dose dependent parameters C_{max} and AUC were normalized for course 3 by multiplying them by the ratio of dose 1/dose 3.

Results

The basic demographic characteristics of the 23 patients are presented in Table 1. On the first course of therapy, all patients received 600 mg/m^2 CP, 40 mg/2 Mtx and 600 mg/m^2 FUra. For the third course of tretment, some dose adjustments due to toxicity were made such that the mean doses delivered were 585 mg/m^2 CP, 38.8 m^2 Mtx and 585 mg/m^2 FUra. The major pharmacokinetic parameters for all three drugs on courses 1 and 3 are presented in Table 2. A representative plasma concentration-time curve for CP, Mtx and FUra in one patient is presented in Fig. 1. No statistically significant difference in the mean AUC of CP, Mtx or FUra was seen between these two courses. The FUra AUC values on the first course of therapy ranged from 4,840 to 17,390 μM min, whereas the AUC for CP varied between 29,914 and 70,685 μM min and the Mtx

Table 2. Cyclophosphamide, methotrexate and 5-fluorouracil pharmacokinetics on courses 1 and 3[a, b]

Parameter	Cyclophosphamide				Methotrexate				5-fluorouracil			
	Course 1		Course 3		Course 1		Course 3		Course 1		Course 3	
AUC (μM min)	51,955 (11,315)		57,579 (11,655)		1,008 (199)		1,091 (277)		8,689 (3,582)		10,362 (3,788)	
Clearance (ml/min)	78	(20)	69	(14)	153	(29)	143	(32)	1,025 (348)		849	(284)
Mean residence time (min)	457	(87)	504	(115)	204	(43)	211	(46)	21.0	(8.1)	28.2	(9.4)
Peak plasma concentration (μM)	121	(20)	128	(25)	14.3	(3.8)	16.3	(5.2)	481	(264)	655	(277)

[a] Data expressed as mean values (±SD), $n = 23$

[b] Values for course 3 normalized to account for changes in dose between course 1 and course 3

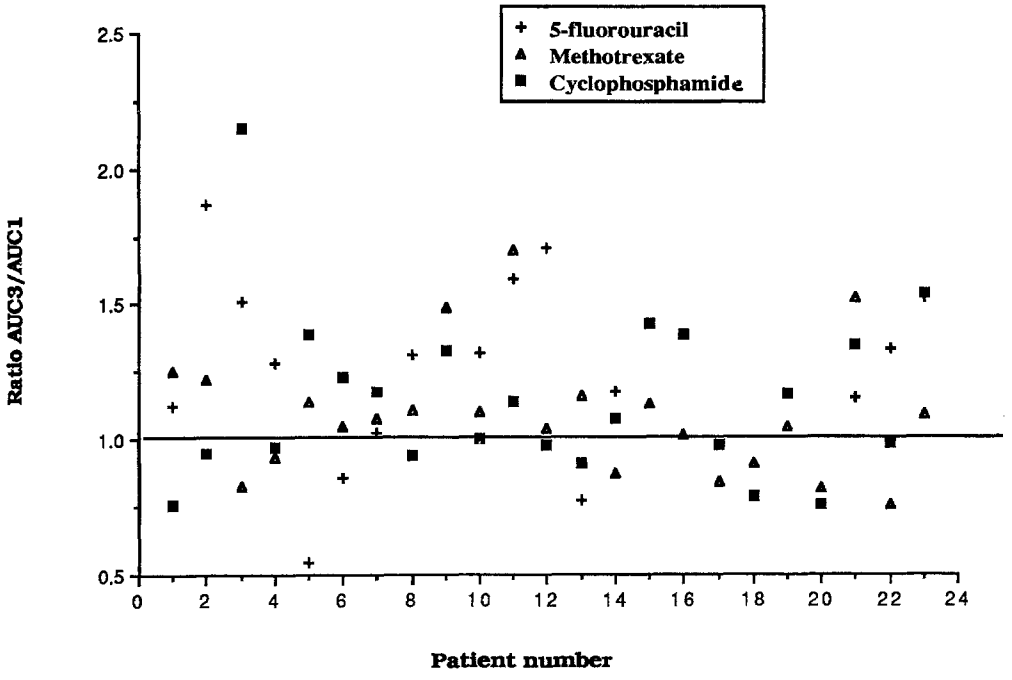

Fig. 2. Intrapatient variability expressed as the ratio of $AUC_{course\ 3}/AUC_{course\ 1}$ for all 3 drugs in each of the 23 patients

AUC ranged from 691 to 1,546 μM min. There was no relationship between the pharmacokinetics of the three drugs, such that having a lower than normal clearance of CP, Mtx or FUra did not predict for having a lower than normal clearance of the other two drugs.

The relationship between renal function (as measured by both plasma creatinine and measured 24-h creatinine clearance) and the total body clearance of all three drugs was examined. The only statistically significant correlation noted was that Mtx clearance on the first course of chemotherapy correlated with 24-h creatinine clearance ($r^2 = 0.42$, $P < 0.01$), but this was not maintained for the third course of chemotherapy ($r^2 = 0.10$, $P > 0.05$). No relationship between plasma bilirubin, AST or ALT values and the clearance of any drug was seen. There was no significant change in creatinine clearance or levels of bilirubin or transaminases between the first and third courses of therapy.

The degree of intraindividual variability was examined by determining the correlation coefficients for AUC in each patient between courses 1 and 3 (with AUC on course 3 being normalized if a change in dose had occurred). There was little correlation in the CP AUC for individual patients ($r^2 = 0.09$, P = not significant), whereas for Mtx and FUra a modest correlation was seen ($r^2 = 0.25$ and 0.21, respectively; $P < 0.05$). In individual patients the AUC for CP on the third course ranged between 76% and 215% of that seen on the first course of therapy; for Mtx the AUC range was 77%–170% and for FUra it was 55%–185% (Fig. 2).

Discussion

The routine use of plasma concentration monitoring in patients receiving systemic treatment for cancer is currently limited to those receiving high-dose Mtx infusions. A more widespread application of these techniques has been limited by a number of factors, including the heterogeneity of response, the use of combination treatments and the complexities of accurately characterizing systemic exposure [16]. Our intent in this study was to perform a detailed examination of CP, Mtx and FUra pharmacokinetics and then explore whether plasma concentration monitoring could be justified to improve the treatment outcome. In any

Table 3. Comparison of inter- and intrapatient variability in drug AUC

Drug	Interpatient – course 1[a]	Intrapatient – courses 1 and 3[b]
Cyclophosphamide	0.58 – 1.36	0.76 – 2.15
Methotrexate	0.69 – 1.53	0.77 – 1.70
5-fluorouracil	0.55 – 1.97	0.55 – 1.87

[a] Expressed as the range of values for $AUC_{course\ 1}$/mean $AUC_{course\ 1}$ for all 23 patients

[b] Expressed as the range of values for $AUC_{course\ 3}/AUC_{course\ 1}$ for all 23 patients

such application this information could not be used on that particular course (as is done in high-dose Mtx monitoring) but rather would be taken into account in determining the drug dose for subsequent treatments. The frequency of blood samples done on our study would not be possible in routine practice, but this could be addressed by the use of limited sampling strategies to estimate the AUC, as has been demonstrated for CP by Egorin et al. [6].

For plasma pharmacokinetic information to be useful clinically, it needs to be demonstrated that there is interpatient variability in drug disposition and that this variability is important in determining efficacy and toxicity. If the information gained from pharmacokinetic monitoring on one course of treatment is to be used to determine the drug doses for subsequent courses, then intrapatient variability should be minimal. Otherwise, patients who have dose escalation based on a low initial systemic exposure could subsequently be at risk for excessive toxicity. Systematic changes in drug disposition that occur due to factors such as enzyme induction or disease progression would also limit the utility of such techniques.

In this study a relatively homogeneous population of women with early breast cancer and normal liver and renal function was chosen so as to minimize the factors that might increase pharmacokinetic variability. Interpatient variability in drug disposition was seen for CP, Mtx and FUra to a degree consistent with reports from other studies [5, 12, 17]. Only minor systematic changes in the average drug disposition parameters for the 23 patients between the first and third course of therapy were detected. The AUC is probably the best pharmacokinetic measure of overall systemic exposure, and it did not change significantly for any of the three drugs.

Although the mean values for drug disposition did not change significantly between the first and third courses of therapy, this does not imply that values remained relatively constant in each patient between the two courses. When paired comparisons of pharmacokinetic parameters were made, the correlation coefficient was always ≤ 0.5 and, in many cases, no statistically significant correlation between course 1 and course 3 could be detected. For any patient given identical therapy 6 weeks apart, the drug AUC on the later course of treatment could vary from approximately one-half to more than twice that seen on course 1. The proportionate range over which the AUC could vary between the two courses of therapy in any individual was comparable with the range of AUC values observed between different patients on a single treatment (Table 3).

The degree of intrapatient variability in drug disposition in cancer patients receiving repeated therapy has not been widely studied. Milano et al. [14] noted a wide intrapatient variability in FUra AUC in patients receiving repeated courses of this drug by intrahepatic infusion that they proposed to be due to changes in tumor mass within the liver. This could not account for the intraindividual variability seen in our study, as no woman had clinical, biochemical or radiological evidence of tumor. A number of investigators are actively examining the relationships between anticancer drug pharmacokinetics and pharmacodynamics. In such studies it has been proposed that drug doses be adjusted in future courses on the basis of the results of plasma pharmacokinetic monitoring. The degree of intrapatient variation in CP, Mtx and FUra pharmacokinetics seen in our study indicates that caution should be used in such dose adjustments unless it has previously been demonstrated that drug disposition remains constant.

Other potential explanations for variability in drug disposition would include inaccuracies in sample collection or analysis, alterations in the function of the metabolic end organs, the use of concomitant medications or the presence of co-existent disease. All samples were collected, processed and labeled by one study nurse, with the exact time of the sample in relation to drug administration being recorded. Mtx was analyzed using a widely available automated method, whereas CP and FUra were measured using well-established HPLC methods in a laboratory under the direct supervision of a clinical biochemist (S.S. and P.B.). The day-to-day coefficient of variation of all three assays was less than 10%. Impairment of renal function may lead to alterations in the disposition of Mtx, but no consistent effect of kidney or liver disease on the disposition of CP or FUra was demonstrated. We could not account for any of the variability in our patients on the basis of differences in renal function or liver function. All women in this study had no other serious illness, and none had evidence of metastatic breast cancer. The use of ancillary medications such as anti-emetics was standardized. Therefore, although this study demonstrated significant variability in drug disposition, we could not identify any factor that might account for this. The factors underlying such variability in pharmacokinetics and the relevance of the variability to the subsequent response need to be addressed in future studies.

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