Anti-Cancer Drugs 1999, **10**, pp. 719–728

Clinical study

Clinical pharmacokinetics of doxorubicin in combination with GF120918, a potent inhibitor of MDR1 P-glycoprotein

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Previous clinical investigations with doxorubicin indicated that modulators of P-glycoprotein dramatically decrease the systemic clearance of the drug, which complicates the interpretation of toxicity and response data. In the present study, we examined the pharmacokinetics of doxorubicin and GF120918, a novel potent P-glycoprotein inhibitor, in cancer patients in a search for more selective modulation of multidrug resistance (MDR). Seven cohorts (46 patients) received sequential treatments with doxorubicin alone by a 5 min i.v. bolus (50–75 mg/m^2), oral GF120918 alone (50 mg q.d.–400 mg b.i.d.), and the combination of doxorubicin and GF120918. Serial blood and urine samples were taken during both treatment courses and analyzed for doxorubicin and its metabolite doxorubicinol by a liquid chromatographic assay. The pharmacokinetic characteristics of doxorubicin in the presence or absence of GF120918 indicate a very minor overall effect of the modulator, except at the highest combined dose level (i.e. 75 mg/m^2 *plus* 400 mg b.i.d.). A limited number of patients experienced significantly increased exposure to doxorubicinol upon combined treatment, which was associated with concomitantly higher plasma levels of GF120918. Sigmoidal maximum-effect models revealed significant correlations ($p < 0.02$) between the area under the curve of doxorubicinol and the percent decrease in neutrophils and platelets. Sigmoidicity factors in the fitted Hill equation were similar between both treatment courses, suggesting no pharmacodynamic potentiation of doxorubicinol myelotoxicity by GF120918. Our data indicate that GF120918 at the tested doses of combination treatment achieves plasma concentrations that reverse MDR in experimental models and it lacks the significant kinetic interaction with doxorubicin observed previously with other modulators. Hence, it may be possible in future trials to assess the contribution of a potent inhibitor of P-glycoprotein activity to the toxicity and activity of doxorubicin with the knowledge that profound plasma pharmacokinetic interactions are unlikely. [© 1999 Lippincott Williams & Wilkins.]

Key words: Doxorubicin, drug resistance, GF120918, P-glycoprotein, pharmacokinetics.

Introduction

Cellular resistance to antineoplastic agents continues to be a major impediment in cancer chemotherapy for many human malignancies. One type of resistance [multidrug resistance, (MDR)], induced by structurally unrelated drugs such as vinca alkaloids, taxanes and anthracyclines, is related to the overexpression of the MDR1 gene and production of a 170 kDa membrane-associated P-glycoprotein.[1] This protein is believed to function as an energy-dependent drug efflux pump for various hydrophobic xenobiotics, resulting in reduced intracellular drug accumulation.[2] Efforts thus far to overcome the phenomenon of MDR have primarily focused on inhibition of P-glycoprotein activity with non-cytotoxic modulators that compete with the binding and/or transport of the substrate drug.[3] Initial attempts to improve therapeutic response in patients with MDR tumors using combination treatment with modulators (e.g. verapamil and cyclosporin A) were only of limited success due to intrinsic toxicity to the patient.[4–8] Recently, modulators have become available, including the *R*-isomer of verapamil and the cyclosporin D analog SDZ PSC-833, that lack the severe toxicity and usually possess higher affinity for P-glycoprotein. However, clinical application of these compounds is problematic due to the occurrence of

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profound pharmacokinetic interactions with the antineoplastic agent, which confound the interpretation of toxicity and response data.[9-12] The vast majority of interactions are characterized by a dramatically decreased systemic drug clearance, resulting in a need to reduce the dose of the antineoplastic agent because of exacerbated toxicity. Based on these findings, it has been suggested that administration of P-glycoprotein modulators is unlikely to improve the therapeutic index of anticancer chemotherapeutics unless such agents lack significant pharmacokinetic interactions.[13] There is thus a clear need for the identification of more selective inhibitors of P-glycoprotein activity.

Recently, preclinical *in vitro* and *in vivo* evaluation of a novel synthetic modulator of P-glycoprotein, GF120918 was described by Hyafil *et al.*[14] GF120918, an acridinecarboxamide derivative, fully reverses P-glycoprotein-mediated resistance *in vitro* at concentrations as low as about 30 ng/ml and restores sensitivity of various MDR tumors to doxorubicin *in vivo*.[14] These features, in conjunction with the avoidance of an adverse pharmacokinetic interaction with doxorubicin in tumor-bearing mouse models,[14] may lead to substantially increased selectivity of MDR modulation at the tumor site and restore sensitivity of resistant tumors in patients. It is noteworthy, however, that subsequent evaluation in dogs found a decrease in the clearance of doxorubicin when GF120918 was administered, resulting in a 28% increase in doxorubicin AUC values. Doxorubicinol AUC values were increased by 78 and 118%, respectively, in male and female beagle dogs.[15] On the basis of these results, we have recently conducted a clinical phase I trial of GF120918 given in combination with doxorubicin to determine the maximum tolerated dose, and qualitative and quantitative toxicities of the combination.[16] GF120918 was administered orally with the intent of achieving steady-state plasma levels of approximately 100 ng/ml.[14] In the present report, we examined the pharmacokinetics of doxorubicin in a group of patients with advanced solid tumors treated in a sequential cross-over design with and without GF120918.

Patients and methods

Study design

The pharmacokinetics of doxorubicin were studied in adult patients participating to a phase I study of the drug given in combination with GF120918 in various non-hematological malignancies.[16] All patients had a histologically confirmed diagnosis of malignant solid tumor for which no standard effective therapy existed and had no evidence of major alterations of hematopoietic, hepatic, cardiac or renal function at the time of the study. All patients had a predicted life expectancy of at least 3 months, an Eastern Cooperative Oncology Group performance status of 0-2 and had no prior chemotherapy with anthracycline or anthracenedione derivatives. Written informed consent was obtained from each individual and the protocol was approved by the institutional ethics committee.

GF120918 was provided by Glaxo Wellcome (Research Triangle Park, NC) as 800 mg tablets containing 25, 100 or 250 mg of the compound as hydrochloride salt, and was given orally with 50-100 ml of water within 1 h after a meal. Doxorubicin, purchased from Adria Laboratories (Columbus, OH), was diluted in sterile isotonic saline and delivered as a 5 min i.v. bolus through a venous catheter, without an in-line filter. Patients were first treated with a single dose of doxorubicin alone (at 50-75 mg/m^2) on day 1 (course 1), then with GF120918 alone (at 50 mg q.d.-400 mg b.i.d.) on days 22-24 (course 2) and finally with GF120918 on days 29-33 in combination with doxorubicin on day 31 (course 3).

Sampling schedule and drug analysis

Blood specimens were acquired in all patients during the first and third courses of treatment. Blood volumes of about 5 ml were drawn directly into Vacutainer plasma tubes containing lyophilized sodium heparin (Becton Dickinson, Meylan, France) from a peripheral venous access device. Samples for anthracycline analysis were collected immediately before treatment, and at 0.08 (end of infusion), 0.5, 1, 2, 4, 7, 12, 24 and 48 h after start of infusion. For GF120918 measurement, blood samples were obtained before, and 1, 2, 4, 7, 12 and 24 h after dosing. All blood samples were centrifuged immediately for 5 min at 3000 *g* to yield plasma, which was stored frozen in polypropylene vials at −80°C until the time of analysis. In a limited number of patients, complete urine collections were obtained for 24 or 48 h after start of drug administration.

Plasma and urine concentrations of doxorubicin and doxorubicinol were determined by a validated reversed-phase high-performance liquid chromatographic assay described elsewhere.[17] In brief, quantitative extraction was achieved by a single protein-precipitation step of 1 ml samples with 500 μl of acetone in the presence of 100 μl of aqueous zinc sulfate. The compounds of interest, including the

internal standard daunorubicin, were separated isocratically at 50°C on a column packed with Inertsil ODS-80A material (LC-Service, Emmen, The Netherlands) and a mobile phase composed of water: acetonitrile:tetrahydrofuran (76:24:0.5, v/v/v; pH 2.0). The mobile phase was delivered at a flow rate of 1.25 ml/min, and the column effluent was monitored fluorimetrically at an excitation wavelength of 480 nm and an emission wavelength of 560 nm. Detection and integration of chromatographic peaks was performed by the Fisons ChromCard data analysis system, connected to an ICW workstation (Milan, Italy). With each analytical run, duplicate calibration curves were prepared in drug-free plasma at concentrations ranging from 0.50 to 100 ng/ml, and analyzed together with patient samples and a set of quality control samples spiked at four different concentrations. Calibration curves were computed using the ratio of the peak height of the analytes and the internal standard versus the nominal concentration (x), using weighted ($1/x^2$) least-squares linear-regression analysis. The percent deviation from nominal values, and the between-run and within-run variation at the various concentration levels for each compound were always below 10%. Blood samples collected and processed to plasma were also analyzed for GF120918 using a validated chromatographic assay preceded by single solvent extraction as described.[18]

Pharmacologic data analysis

Individual patient plasma concentration-time data were analyzed using the Siphar software package (version 4.0; SIMED, Créteil, France), by determination of slopes and intercepts of the plotted curves with multi-exponential functions. Initial parameter estimates were determined by the program and improved using an iterative numerical algorithm based on Powell's method.[19] Model discrimination was assessed by a variety of considerations, including visual inspection of the predicted curves, dispersion of residuals, minimization of the sum of weighted squares residuals, and the Akaike[20] and Schwarz[21] information criteria. In all cases, concentration-time profiles of doxorubicin and doxorubicinol were best simultaneously fitted to a bi-exponential equation after zero-order input with weighting according to y_{obs}^{-1}. Final values of the iterated parameters of the best-fit equation were used to calculate pharmacokinetic parameters using standard equations.[22]

Pharmacokinetic-pharmacodynamic relationships were evaluated using the Siphar and NCSS (version 5.X; Dr Jerry Hintze, Kaysville, UT) programs, using linear and maximum-effect modeling. The observed models were rated for goodness of fit by minimization of sums of squared residuals and by reduction of the estimated coefficient of variation for fitted parameters. Significance of the relationships was assessed by construction of contingency tables with subsequent χ^2 analysis.

Statistical considerations

Pharmacokinetic parameters for doxorubicin and doxorubicinol are reported as mean ± SD. Variability

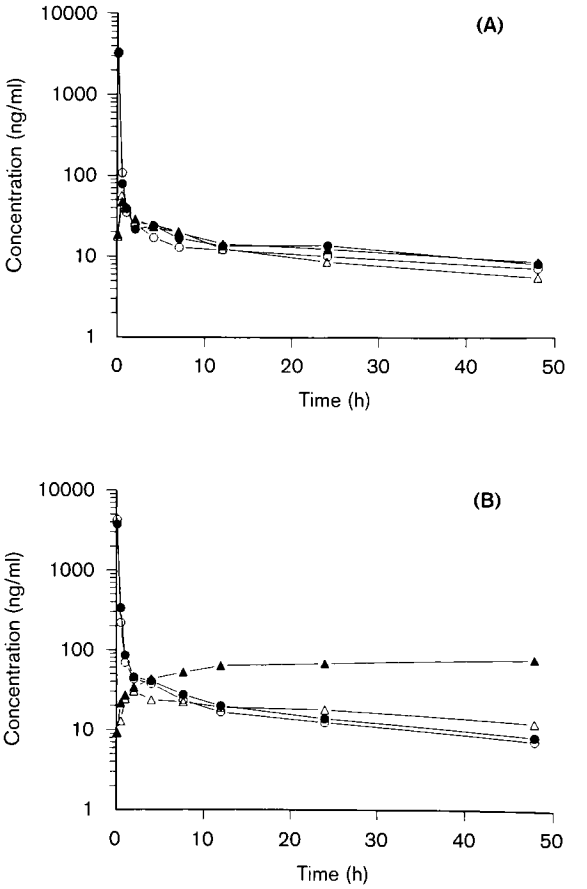

Figure 1. Representative plasma concentration versus time curves of doxorubicin (circles) and its metabolite doxorubicinol (triangles) in two patients treated with doxorubicin alone (open symbols) or in combination with oral GF120918. The patient displayed in (A) received 50 mg/m^2 of doxorubicin with or without 100 mg b.i.d. of oral GF120918, whereas the patient displayed in (B) received 50 mg/m^2 of doxorubicin with or without 400 mg b.i.d. of oral GF120918. Note the effect of GF120918 on the plasma concentrations of doxorubicinol in the latter patient, which are still increasing at the last sampling time point.

Table 1. Plasma pharmacokinetic parameters of doxorubicin and doxorubucinol in the absence or presence of GF120918[a]

Cohort[b]	Doxorubicin						Doxorubicinol	
	$AUC_{0-\infty}$[c] (μg.h/ml)	C_{max} (μg/ml)	$t_{½(\alpha)}$ (h)	$t_{½(\beta)}$ (h)	V_d (l/m)	MRT (h)	$AUC_{0-\infty}$ (μg.h/ml)	C_{max} (ng/ml)
1 ($n=5$)								
without GF	1.67±0.353	3.05±1.23	0.13±0.04	10.6±1.86	402±77	7.51±1.84	0.539±0.331	33.2±11.7
with GF	1.55±0.361	2.97±0.91	0.11±0.04	10.3±3.34	460±99	8.19±3.09	0.580±0.286	45.6±16.6
2 ($n=4$)								
without GF	1.33±0.414	2.13±1.09	0.12±0.05	9.87±3.08	550±269	7.97±3.58	0.416±0.175	52.4±33.3
with GF	1.31±0.281	1.89±1.10	0.14±0.05	11.1±1.88	779±278	12.11±3.52	0.695±0.319	46.8±27.9
3 ($n=5$)								
without GF	1.66±0.566	3.24±0.61	0.10±0.01	12.9±3.55	595±188	8.79±3.03	0.506±0.136	41.2±14.4
with GF	1.62±0.352	3.25±1.02	0.08±0.01	12.5±1.98	650±149	10.1±1.60	0.635±0.228	47.8±15.8
4 ($n=5$)								
without GF	1.26±0.296	3.70±1.02	0.09±0.01	9.70±1.39	798±293	7.86±2.74	0.493±0.199	37.8±15.4
with GF	1.62±0.363	3.64±1.03	0.09±0.01	10.7±3.91	488±319	7.03±2.15	0.738±0.322	46.0±18.6
5 ($n=5$)								
without GF	1.39±0.327	2.49±0.38	0.09±0.02	14.0±4.53	733±260	11.5±6.11	0.651±0.323	37.8±6.49
with GF	1.72±0.324	3.56±1.71	0.12±0.07	16.8±4.53	932±390	15.3±7.17	1.35±0.648§	55.0±14.8[d]
6 ($n=4$)								
without GF	1.85±0.245	3.66±0.97	0.09±0.02	14.2±1.60	762±207	15.5±2.72	0.743±0.058	25.8±2.59
with GF	1.99±0.233	3.93±1.55	0.09±0.02	14.1±4.00	725±151	12.0±3.25	0.969±0.244	32.5±5.17
7 ($n=4$)								
without GF	1.70±0.398	3.11±1.40	0.10±0.01	14.2±1.05	1040±254	13.6±2.00	0.810±0.201	38.5±7.70
with GF	2.57±0.295[d]	3.90±0.77	0.10±0.01	18.8±3.02	983±187	18.6±6.60	2.11±0.911[d]	55.5±19.9

[a]Data were obtained from cancer patients after the first (without GF120918) and third treatment course (with GF120918) of a rapid bolus infusion of doxorubicin. The kinetic terms are mean values±SD.

[b]Cohort 1, 50 mg/m^2 of doxorubicin±50 mg q.d. of GF120918; cohort 2, 50 mg/m^2 of doxorubicin±50 mg b.i.d. of GF120918; cohort 3, 50 mg/m^2 of doxorubicin±100 mg b.i.d. of GF120918; cohort 4, 50 mg/m^2 of doxorubicin±200 mg b.i.d. of GF120918; cohort 5, 50 mg/m^2 of doxorubicin±400 mg b.i.d. of GF120918; cohort 6, 60 mg/m^2 of doxorubicin±400 mg b.i.d. of GF120918; cohort 7, 75 mg/m^2 of doxorubicin±400 mg b.i.d. of GF120918.

[c]Abbreviations: $AUC_{0-\infty}$, area under the plasma concentration versus time curve extrapolated to infinity; C_{max}, maximum plasma concentration of drug; $t_{½(i)}$, half life of the i-th disposition phase; V_d, volume of distribution; MRT, mean residence time; n, number of patients evaluated at both treatment courses; GF, GF120918.

[d]$p<0.05$ versus doxorubicin treatment alone (paired Student's t-test).

in parameters between the various doxorubicin dose levels was evaluated by the Kruskal-Wallis statistic followed by a Dunn's test to determine which group differed. Interpatient differences in kinetics were assessed by the coefficient of variation, expressed as the ratio of the SD and the observed mean. To test parameter differences for statistical significance among treatment courses, a two-tailed paired Student's *t*-test was performed. The correlation between peak plasma concentrations of GF120918 and the administered dose level or the corresponding AUC ratio (course 3/course 1) observed for doxorubicin and doxorubicinol were analyzed by means of Spearman's or Pearson's correlation coefficient, respectively, and linear regression analysis. Probability values of less than 0.05 were regarded as statistically significant. All statistical calculations were performed using NCSS and STATGRAPHICS *Plus* (version 2; Manugistics, Rockville, MA).

Results

Patient characteristics

Forty-six patients were entered on the trial to receive sequential treatments with a single i.v. bolus dose of doxorubicin (course 1), 3 days of oral GF120918 (course 2), and the combination of GF120918 and doxorubicin (course 3). The entire group consisted of 26 males and 20 females ranging in age from 25 to 78 years, with a median age of 51 years. Each patient had a malignant solid tumor with colorectal cancer (n=13), ovarian (n=8) and renal (n=6) cancer being the predominant disease types. At a fixed dose of doxorubicin at 50 mg/m^2, GF120918 doses were escalated in cohorts of patients receiving 50 mg q.d. (n=6), 50 mg b.i.d. (n=5), 100 mg b.i.d. (n=5), 200 mg b.i.d. (n=5) and 400 mg b.i.d. (n=11). The target GF120918 mean steady-state level of 100 ng/ml or greater was achieved with a drug dose of 400 mg b.i.d. At this dose, the dose of doxorubicin was further escalated to 60 (n=8) and 75 (n=6) mg/m^2. Full clinical toxicities and treatment responses will be reported in detail elsewhere. For the evaluation of doxorubicin pharmacokinetics and pharmacokinetic-pharmacodynamic relationships, we included only the patients who had sampling and complete pharmacokinetic data during both courses 1 and 3 (n=37). Some patients with missing data at essential time points, e.g. at the end of the 5 min infusion, were also excluded from the analyses (n=4). Eventually, 33 out of 46 patients were evaluable for pharmacologic analysis.

Doxorubicin pharmacokinetics

The plasma concentration-time profiles for doxorubicin and doxorubicinol with and without GF120918 co-treatment were similar for all patients studied. In both courses of treatment, disposition phases of doxorubicin and doxorubicinol exhibited a biexponential decay and could be fitted to a two-compartment model (Figure 1A), as described previously.[23] The mean pharmacokinetic parameters of doxorubicin and doxorubicinol for both study courses are summarized as a function of the treatment cohort in Table 1. The doxorubicin total body clearance and the area under the curve (AUC) ratio of doxorubicinol and doxorubicin across all dose levels without GF120918 co-treatment were 1264±369 ml/min (mean±SD range: 637-2312 ml/min) and 0.381±0.07 (mean±SD, range: 0.301-0.476), respectively. These values are consistent with previously published values obtained with doxorubicin used as a single agent.[24-27] Overall, the pharmacokinetic characteristics of doxorubicin and doxorubicinol in the presence of GF120918 indicate a very minor effect of the modulator. In fact, statistically significant differences between study courses (as evaluated by paired Student's *t*-test) for any kinetic parameter were observed only for doxorubicinol in cohort 5 (i.e. 50 mg/m^2 of doxorubicin *plus* 400 mg b.i.d. of GF120918), and for both doxorubicin and doxorubicinol in cohort 7 (i.e. 75 mg/m^2 of doxorubicin *plus* 400 mg b.i.d. of GF120918). The increase in doxorubicinol exposure,

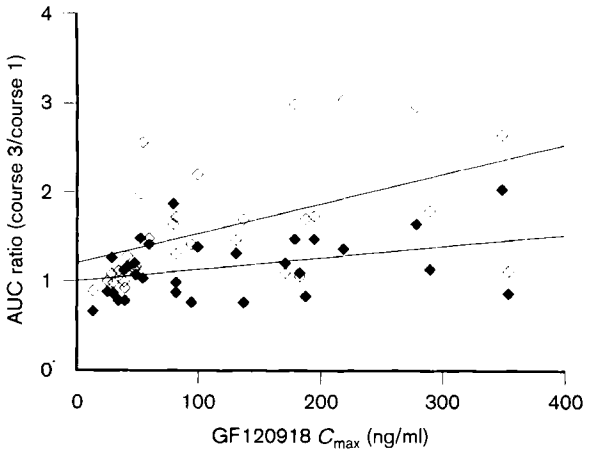

Figure 2. Linear relationships between the C_{max} of GF120918 and the ratio of the AUC obtained with (course 3) and without co-treatment with GF120918 (course 1) of doxorubicin (closed symbols; r=0.375; p=0.041) and doxorubicinol (open symbols; r=0.499; p=0.005).

as measured by the AUC, in cohort 5 reaches only borderline significance (p=0.049) and was mainly caused by data from two out of five patients displaying more than 3-fold increase in the AUC (see, e.g. Figure 1B). Analysis of potentially interfering co-medication could not account for the observed differences in AUC values between patients and between study courses (not shown), suggesting that this phenomenon is GF120918 related. To gain further insight into this hypothesis, we have also analyzed the ratios of the AUCs of doxorubicin and doxorubicinol in courses 3 and 1 as a function of the maximum plasma concentration of GF120918 in individual patients (Figure 2). These data indicate that there is only a minimal, albeit significant, overall increase of the ratio of doxorubicin in the presence of GF120918 (Pearson's r=0.375; p=0.041), whereas the ratio of doxorubicinol is more clearly and significantly dependent on the GF120918 plasma concentrations (Pearson's r=0.499; p=0.005). Furthermore, the kinetic interaction between doxorubicinol (but not doxorubicin) and GF120918 was significantly more pronounced in patients with maximum GF120918 plasma concentrations above 80 ng/ml [p=0.011 (doxorubicinol) versus p=0.328 (doxorubicin)] (Figure 3).

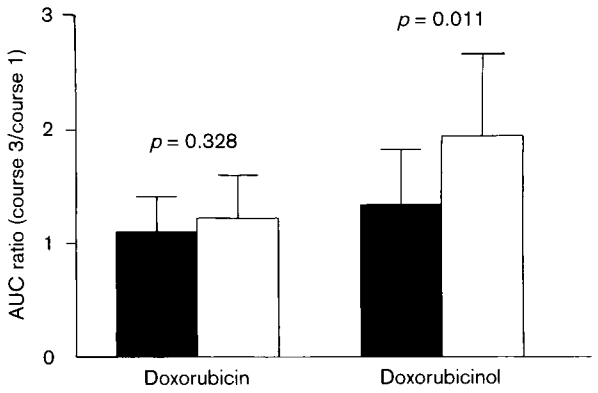

Figure 3. AUC ratios (course 3/course 1) of doxorubicin and doxorubicinol as a function of the C_{max} of GF120918 in cohorts of patients with concentrations below 80 ng/ml (black bars, n=18) or concentrations above 80 ng/ml (white bars, n=13).

The cumulative urinary excretion and renal clearance, defined as the amount excreted divided by the plasma AUC during the sampling period, of doxorubicin and doxorubicinol in patients receiving 400 mg b.i.d. of GF120918 is shown in Table 2. Overall, the excretion of doxorubicin was equal in both study courses, whereas that of doxorubicinol was significantly higher in course 3 (p=0.020). The renal clearance of doxorubicinol, however, was not significantly different in both courses, indicating that GF120918 lacks a direct quantitative effect on (P-glycoprotein-mediated) urinary excretion that might explain the increased systemic exposure to this metabolite.

Doxorubicin pharmacodynamics

Pharmacokinetic-pharmacodynamic relationships between the AUCs of doxorubicin and doxorubicinol and hematologic toxicity (white blood cells, neutrophils and platelets) were evaluated using linear, log-linear and sigmoidal maximum-effect models. For toxicity, absolute values, toxicity gradings, percent of decrease in cell count and survival fractions[28] were considered. We found no significant correlation between the AUC of doxorubicin and myelotoxicity using any model

Table 2. Elimination kinetics of doxorubicin and doxorubicinol in the absence or presence of 400 mg b.i.d. of GF120918[a]

Parameter	Without GF120918	With GF120918
Doxorubicin		
CL_T (ml/min)[b]	1220±348 (637–1870)	1050±203 (703–1520)
CL_R (ml/min)	82.4±36.7 (27.4–145)	60.6±25.8 (12.0–101)
CL_{ER} (ml/min)	1140±333 (609–1800)	892±187 (692–1430)
fe_u (%)	6.80±2.62 (3.10–11.0)	6.24±2.36 (1.70–9.50)
Doxorubicinol		
CL_R (ml/min)	50.9±18.5 (28.0–87.7)	47.0±19.7 (16.3–81.7)
fe_u (%)	1.85±0.62 (1.00–3.30)	2.65±0.62 (1.70–3.80)[c]

[a]Data were obtained from 12 cancer patients after the first (without GF120918) and third treatment course (with 400 mg b.i.d. of GF120918) of a rapid bolus infusion of doxorubicin at dose levels ranging from 50 to 75 mg/m^2. The kinetic terms are mean values±SD, with range in parentheses.

[b]Abbreviations: CL_T, total clearance; CL_R, renal clearance; CL_{ER}, extrarenal clearance; fe_u, percent of the absolute doxorubicin dose excreted in urine as indicated drug.

[c]$p<0.05$ versus doxorubicin treatment alone (paired Student's t-test).

($p \geq 0.058$). The AUC of doxorubicinol, however, was significantly correlated (using the sigmoidal maximum-effect model) with the percent decrease in absolute neutrophil count (r=0.53, p=0.002 for course 1; r=0.58, p=0.001 for course 3) and the percent decrease in platelet count (r=0.39, p=0.024 for course 1; r=0.57, p=0.001 for course 3), but not with white blood cell counts (p=0.43) (Figure 4).

Discussion

In recent years, numerous trials have been performed to determine the feasibility of adding P-glycoprotein modulators to chemotherapeutic agents administered to patients. The idealized putative clinical benefit is that their use will increase the proportion of patients responding with antitumor activity, without affecting the dose-toxicity curve (i.e. by increasing the therapeutic index). Given the overlap in specificity of enzymes responsible for the metabolism of both anticancer drug and modulator, and the clinical data indicating that drug interactions are likely, it appears that increased toxicity can be anticipated based on a pharmacokinetic effect alone, thereby narrowing the therapeutic dosage range for anticancer drugs.[13] For example, *R*-verapamil, cyclosporin A and the related compound SDZ PSC-833 have been shown to have a profound effect on the pharmacokinetics of etoposide, paclitaxel and doxorubicin, even when the dose of the anticancer drug was reduced by 50%.[7,10–12] The use of the latter two modulators also raises questions as to the specificity of the modulator for P-glycoprotein, as both compounds have been demonstrated to inhibit a bile-acid transporter in the canalicular membrane.[29–31] In addition, the formulation vehicle for i.v. use of cyclosporins (i.e. Cremophor EL) in itself dramatically affects the pharmacokinetic behavior of paclitaxel,[32] doxorubicin[33] and etoposide,[34] thereby potentially non-selectively increasing the incidence or severity of toxic adverse effects.

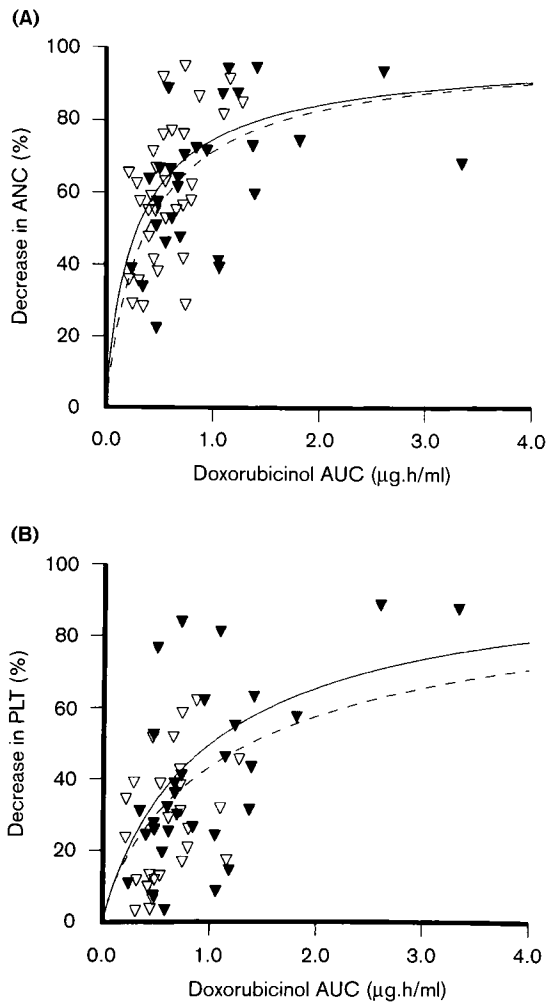

Figure 4. Sigmoidal maximum-effect modeling of the AUC of doxorubicinol and the percent decrease in (A) absolute neutrophil count (ANC) and (B) platelet count (PLT) in patients treated with doxorubicin alone (open symbols, dotted lines) or in combination with oral GF120918 (closed symbols, solid lines).

In the present study we have described the effects of oral administration of the potent inhibitor of P-glycoprotein activity, GF120918, on the clinical pharmacokinetics and pharmacodynamics of doxorubicin, in an attempt to search for more selective modulators of MDR. Plasma levels of the modulator capable of complete reversal of a high level of doxorubicin resistance in cell cultures (about 30 ng/ml) were achieved in all patients receiving GF120918 doses of higher than 100 mg b.i.d. In addition, ongoing experiments based on surrogate marker assays with $CD56^+$ lymphocytes and ^{99m}Tc sestamibi imaging provided data to support our premise of significant P-glycoprotein inhibition by GF120918 in these patients (to be published elsewhere). The results of this clinical study indicate that at the tested doses of combination treatment, GF120918 has only a very minor effect on the pharmacokinetic behavior of the anthracycline, which was characterized by a slight decrease in the systemic clearance, while a more marked effect on doxorubicinol pharmacokinetics was observed in some patients (with higher GF120918 plasma concentrations). Similar findings of a more modest increase in doxorubicin AUC and a more pronounced increase in doxorubicinol AUC in the presence of GF120918 have been reported in dogs.[15] The decrease in doxorubicinol clearance in our

patients was linearly related to individual peak plasma levels of GF120918, implying that further increases in the GF120918 doses, with concomitantly higher concentrations, might induce larger differences in the AUCs of doxorubicin and doxorubicinol between study courses. Additional clinical and pharmacokinetic information is being collected in ongoing trials of this combination to further explore this relationship.[27]

The mechanisms responsible for the increased exposure to doxorubicinol observed in some patients when given doxorubicin in combination with GF120918 remain unknown. It is of importance to note, however, that in these patients (with GF120918 plasma concentrations above 80 ng/ml) doxorubicinol concentrations were still increasing at the last time at which concentrations were available (usually 48 h after dosing), similar to studies describing combination treatment of doxorubicin and cyclosporin A or SDZ PSC-833,[8,12] suggesting that the increased systemic exposure results primarily from the interaction of GF120918 with elimination pathways of this metabolite. This is also supported by the fact that only a 1.4-fold higher maximum plasma concentration of doxorubicinol was observed in the presence of GF120918 (cohort 7; Table 1), whereas the corresponding AUC increased by a factor of 2.6. In the same cohort of patients, renal clearance of doxorubicinol was not significantly altered by GF120918, consistent with GF120918 altering extrarenal elimination routes of doxorubicinol. These may involve inhibition of endogenous P-glycoprotein expressed in the bile canaliculi and intestinal epithelial cells, thereby interfering with biliary excretion and modulating re-absorption from the intestinal lumen. However, given the similarity in the chemical structures of the parent drug and its metabolite, it is unlikely that these processes affect doxorubicinol to a greater degree than doxorubicin. Furthermore, preclinical pharmacologic studies performed in mice lacking *mdr1a* P-glycoprotein have shown only slight alterations of the plasma pharmacokinetics and excretion of doxorubicin and metabolites,[35] which correspond well with the presently observed differences between study courses in patients. Enhancement of doxorubicin metabolism by GF120918 leading to higher levels of the metabolite seems also unlikely given the sustained plasma levels of the parent drug in the presence of GF120918. At present, the most reasonable explanation for the increased exposure to doxorubicinol in patients treated in the final cohorts with GF120918 appears impairment of (hepatic) doxorubicinol metabolism by inhibiting reductive NADPH glycosidase and/or cytochrome P-450-mediated demethylation.

Clinically, we observed that addition of GF120918 resulted in more pronounced neutropenia and leukocytopenia during combination treatment in the patients with increased exposure to doxorubicinol. This finding is supported by the significant relationships observed between the AUC of doxorubicinol and the decreases in neutrophil and platelet count. Interestingly, such relationships were not observed for doxorubicin, which is more cytotoxic than the metabolite and is in general considered responsible for (severe) myelotoxicity.[8] As observed from the raw data in a scattered plot, the lack of significant associations between the AUC of doxorubicin and myelotoxicity seems to be primarily related to insufficient data points in the lower (below 1.0 μg.h/ml) and higher (above 3.0 μg.h/ml) AUC regions. This may, in turn, relate to both a minor inter-patient variability in pharmacokinetics of the parent drug (coefficients of variation: 21.5 versus 42.5% for doxorubicinol) and the relative lack of effect of GF120918 co-treatment on the AUC. It is noteworthy, that the AUC predicted to result in half of the maximum response (AUC_{50}) and the sigmoidity factor [or Hill constant (γ)] in the fitted equations in cases of significant relationships with doxorubicinol were very similar between the study courses. This similarity was particularly striking in the case of the association between the AUC of doxorubicinol and the percent decrease in absolute neutrophil count in study courses with GF120918 (AUC_{50}=0.29 μg.h/ml, γ=0.88) and without GF120918 (AUC_{50}=0.32 μg.h/ml, γ=0.96) (Figure 2A), suggesting no pharmacodynamic potentiation of doxorubicinol myelotoxicity by the modulator. This is also evident from the lack of any correlation in the population studied between the change in the ratio of neutrophil survival fraction in courses 3 and 1, and the percent increase in the AUC of doxorubicinol induced by GF120918 (not shown).

In conclusion, our present data indicate that GF120918 at the tested doses of combination treatment achieves plasma concentrations that fully reverse MDR in experimental models and that it lacks the significant pharmacokinetic interaction with doxorubicin observed previously with other modulators. Hence, it may be possible in future trials to assess the contribution of a potent inhibitor of P-glycoprotein activity to the toxicity and activity of doxorubicin with the knowledge that profound plasma pharmacokinetic interactions are unlikely.

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(*Received 18 May 1999; accepted 1 July 1999*)