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ORIGINAL ARTICLE

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Interferon-gamma pharmacokinetics and pharmacodynamics in patients with colorectal cancer

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Abstract *Purpose*: The study objectives were to define subcutaneous (s.c.) interferon gamma (IFN-γ) disposition in patients with gastrointestinal malignancies receiving 5-fluorouracil (5-FU) and leucovorin (LV) and to examine the relationship between IFN-γ exposures and Fas upregulation in vivo and in vitro. *Methods*: Patients received IFN-γ (10, 25, 50, 75, and 100 $\mu g/m^2$) with LV and 5-FU, and serial samples were collected after the first dose. IFN-γ concentrations were measured by ELISA. A linear one-compartment model with a lag was fitted to the IFN-γ plasma concentration-time data. To examine the relationship between IFN-γ systemic exposure and biological activity in vivo, cell surface Fas upregulation was assessed in peripheral blood mononuclear cell (PBMC) subcompartments. *Results*: The median (range) apparent IFN-γ clearance was 46 l/m^2 per hour (2.6–92 l/m^2 per hour). With increasing IFN-γ dosages, the area under the concentration-time curve ($AUC_{0\rightarrow\infty}$) and C_{max} increased; however, significant interpatient variability was observed. IFN-γ $AUC_{0\rightarrow\infty}$ and time above 33.3 pg/ml significantly correlated with Fas upregulation in several PBMC compartments, but dosage was significantly correlated with this pharmacodynamic marker only in $CD4^+$ and $CD56^+$ cells. In vitro studies in HT29 cells demonstrated that clinically relevant IFN-γ concentrations (1 to 10 U/ml for 6.5 h) with 5-FU/LV upregulated Fas expression 3.5-fold, similar to that in PBMC in vivo. *Conclusions*: We characterized IFN-γ disposition and developed a limited sampling model for use in future pharmacokinetic studies. Our results showed that IFN-γ upregulates Fas in PBMC in vivo and in HT29 cells in vitro at tolerable, clinically relevant exposures and that monitoring IFN-γ pharmacokinetics/pharmacodynamics may be warranted in IFN-γ clinical use.

Keywords IFN-γ · Pharmacokinetics · Pharmacodynamics · Fas

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Introduction

We have demonstrated that interferon gamma (IFN-γ, Actimmune) upregulates Fas (Apo-1; CD95) expression and potentiates 5-fluorouracil/leucovorin (5-FU/LV) antitumor activity [7, 8, 18]. Human IFN-γ is a cytokine produced by sensitized T lymphocytes in response to specific antigens [1]. The endogenous cytokine is a heterogeneous 166 amino acid polypeptide product of a single gene. Monomeric recombinant IFN-γ purified from *E. coli* is a 140 amino acid polypeptide with a molecular weight of 16,465 Da, which has been shown to regulate apoptosis, mediated by an increase in the expression of Fas on the cell surface [2].

In previous studies of IFN-γ pharmacokinetics small patient populations have been investigated and usually only report limited pharmacokinetic data have been reported [5, 9, 17]. Recently we have completed a phase I trial of IFN-γ in combination with the Mayo Clinic 5-FU and LV regimen in patients with colorectal cancer [15]. In that study we determined limited IFN-γ pharmacokinetic values (i.e., AUC and C_{max}) at the maximum tolerated dose (MTD). Thus, in the present report we provide a comprehensive analysis of the pharmacokinetic data collected at all dosage levels studied in the phase I clinical trial.

Although we have reported in vitro IFN-γ concentrations associated with Fas upregulation [18], the in vivo IFN-γ exposure associated with upregulation and potentiation of Fas in the context of 5-FU/LV chemotherapy is largely unknown. A further objective was to evaluate the in vivo pharmacodynamic response to IFN-γ in patients with gastrointestinal malignancies receiving 5-FU and LV by measuring Fas upregulation in specific cellular populations within peripheral blood mononuclear cells (PBMC). Lastly, we assessed the in vitro Fas upregulation in the HT29 human colon carcinoma cell line at clinically relevant IFN-γ systemic exposures.

Patients and methods

Patients

Patients more than 18 years of age with stage IV colorectal, gastric, pancreatic, small intestinal, esophageal, or gallbladder cancer were candidates for this pharmacokinetic study. Other eligibility criteria included a life expectancy of at least 12 weeks, no chemotherapy within 4 weeks of treatment with adequate recovery from toxic effects, and an Eastern Cooperative Oncology Group (ECOG) performance status 0–2. Patients also had adequate hematological, hepatic, and renal function, no chronic diarrhea (e.g., more than five bowel movements per day), no prior exposure to IFN-γ, and no active, serious infection. The Patient Participation Committee of Baptist Memorial Hospital and the St. Jude Children's Research Hospital Institutional Review Board approved the protocol, and informed, written consent was obtained from each patient according to federal and institutional guidelines.

Drug formulation and administration

IFN-γ was supplied as Actimmune (InterMune Pharmaceuticals, Palo Alto, Calif.) in a sterile, clear, colorless solution filled in a single-dose vial for subcutaneous injection. Each 0.5 ml of Actimmune contained 100 μg (3 MU or 2 MIU) of interferon γ-1b formulated in 20 mg mannitol, 0.36 mg sodium succinate, 0.05 mg polysorbate 20 and sterile water for injection. IFN-γ was administered subcutaneously in the arm.

IFN-γ treatment protocol and dosage

Each cycle of therapy lasted 28 days during which patients received IFN-γ subcutaneously, and 5-FU and LV intravenously. IFN-γ was given on days 1, 3, and 5 of a 28-day cycle with 5-FU and LV given days 1–5 of the 28-day cycle. After IFN-γ administration patients received a LV infusion over 15 min at 200 mg/m^2 followed by 5-FU 370 mg/m^2 by rapid bolus daily, days 1–5 [6]. Patients were sequentially enrolled at the following IFN-γ dosage levels: 10, 25, 50, 75, and 100 μg/m^2. No intrapatient dose escalation was permitted.

Sample collection, preparation, and analysis

The IFN-γ disposition was determined after dose one of the first course of therapy. Blood samples (5–7 ml) were obtained prior to drug administration and then at 30 min, and 1, 1.5, 2, 4, 8, 24 and 48 h after IFN-γ administration. Venous blood samples were collected into K_3EDTA tubes and immediately centrifuged at 800 *g* for 10 min at 4°C to separate plasma. Plasma samples were stored at –20°C until analysis.

IFN-γ determination by ELISA was conducted according to the manufacturer's instructions (R&D Systems, Minneapolis, Minn.) with the exception that each point in the standard curve was prepared individually rather than serially. Thawed plasma (200 μl) was added to a microplate precoated with a monoclonal antibody to IFN-γ and incubated at room temperature for 2 h. Plasma samples were then incubated for 2 h at room temperature with a polyclonal antibody to IFN-γ, which was coupled to horseradish peroxidase. A peroxide solution was added to initiate a colorigenic reaction, which was stopped with 2 *N* sulfuric acid. The concentration of IFN-γ in each specimen was determined based on a standard curve calculated by a four-parameter logistic fit with a range of 4 to 1000 pg/ml by measuring the absorbance at 450 nm with wavelength correction at 540 nm. The limit of quantification of the assay was 4 pg/ml. Because all samples obtained prior to the administration of IFN-γ were below the limit of quantification, endogenous IFN-γ does not significantly interfere with this pharmacokinetic study.

Pharmacokinetic analysis

A one-compartment model with a lag and first-order absorption was fitted to IFN-γ concentration-time data using maximum *a posteriori* (MAP)-Bayesian as implemented in ADAPT II [3]. Parameters for the pharmacokinetic model consisted of the initial lag in absorption (T_{lag}), linear absorption rate constant (K_a), and linear elimination rate constant (K_e). Data sets from 13 patients with complete IFN-γ concentration-time profiles were initially fitted by maximum-likelihood estimation as implemented in ADAPT II [3]. The mean and standard deviation were calculated for each parameter, and these parameter estimates were used as the revised initial estimates for another maximum-likelihood estimation. The mean and standard deviation from the maximum likelihood estimation of K_e, V, K_a, and T_{lag} (0.129 ± 0.077 h^{-1}, 348.6 ± 148.9 l/m^2, 0.49 ± 0.70 h^{-1}, and 0.79 ± 0.38 h, respectively) were used to establish population priors for use in a Bayesian analysis. Then all sets of data, including the data from the 13 patients used to construct the covariance matrix and from 6 additional patients, were modeled using a MAP-Bayesian approach to refine the estimates further and update the covariance matrix. Each observation was assessed for goodness of fit by an estimate of variance for the predicted values. Calculated pharmacokinetic parameters included area under the plasma concentration-time curve from zero to infinity ($AUC_{0 \rightarrow \infty}$), maximum IFN-$\gamma$ concentration (C_{max}), and time of maximum IFN-γ concentration (T_{max}). Pharmacokinetic parameters were summarized using descriptive statistics with the median and range reported.

Development of limited sampling model for IFN-γ

To continue IFN-γ pharmacokinetic studies in future phase II clinical trials we used data from this study to develop a limited sampling model (LSM). As described earlier serial plasma samples were obtained over a period of 48 h, and using maximum likelihood (ADAPT II) individual pharmacokinetic parameters were estimated. From these individual estimates population pharmacokinetic parameters were calculated. We used a variation of D-optimality which uses the Fisher Information Matrix for the MAP estimator. This method accounts for the known distribution of the pharmacokinetic parameters and allows the prior distribution of the pharmacokinetic parameters to influence the sampling scheme and thus fewer samples than model parameters can be used. This

method was implemented in MATLAB (The MathWorks, Natick, Mass.). Due to logistical considerations, we constrained the sampling scheme to two samples prior to 8 h and two samples after 24 h.

Validation of limited sampling model for IFN-γ

To validate the LSM we obtained parameter estimates using MAP-Bayesian (ADAPT II) from the 13 patients with complete data sets. These parameter estimates were compared to the parameter estimates determined from the simulated concentrations at the times in the limited sampling scheme. We determined percent accuracy and bias for K_e, V, K_a, and T_{lag} according to standard methods [16]. In particular:

$$\%\ \text{accuracy} = \sum_{i=1}^{n} \frac{\left|x^i_{actual} - x^i_{estimate}\right|}{x^i_{actual}} / n$$

$$\%\ \text{bias} = \sum_{i=1}^{n} \frac{\left(x^i_{actual} - x^i_{estimate}\right)}{x^i_{actual}} / n$$

where x_{actual} represents the value of the pharmacokinetic parameter using all available samples and $x_{estimate}$ represents the estimated value of the pharmacokinetic parameter using only the set of limited samples. The method was considered unbiased if the %bias did not significantly differ from zero (based on the exact Wilcoxon signed-ranks test) and accurate if the %accuracy was not significantly greater than 10% (based on the exact Wilcoxon signed-ranks test).

Conversion between different systems of IFN-γ units

The units used to express IFN-γ dosage and plasma concentrations vary contributing to the confusion encountered when interpreting results of pharmacokinetic and pharmacodynamic data. The data from the assay used to determine the concentration-time profile of IFN-γ for the patients in this pharmacokinetic study were generated in terms of picograms per milliliter. Moreover, IFN-γ is dosed in micrograms per meter squared; therefore, we chose to report $AUC_{0 \to \infty}$ and C_{max} in terms of amount of IFN-γ (i.e., picograms per milliliter×hours and picograms per milliliter). However, because IFN-γ is a protein, the specific activity in terms of Units or International Units is an informative parameter and often used with regard to potency or pharmacological effects. Thus we have reported the in vitro IFN-γ concentrations in terms of IFN-γ Units (i.e., Units per milliliter). Furthermore, at least two different systems have been used in the literature to report IFN-γ concentrations and pharmacokinetic parameters (e.g. Units per milliliter and micrograms per milliliter; Units per milliliter×hours and micrograms per milliliter×hours), and this confounds comparison of data.

A further complication in the interpretation of IFN-γ pharmacokinetic and pharmacodynamic data is the recent standardization of IFN-γ specific activity from Units to International Units due to calibration of the IFN-γ pharmaceutical preparation standard to the WHO standard in a potency assay (product information, Actimmune, 2000). Two conversion factors have been published, and they are 100 μg IFN-γ = 2 MIU and 100 μg IFN-γ = 3 MU. Therefore, we recommend for this and future studies to translate pharmacokinetic data (in terms of amount) from clinical trials into Units to interpret pharmacodynamic data. For example, in our study the time IFN-γ concentrations remained above 33.3 pg/ml was converted to 1 U/ml, allowing us to evaluate the effect of IFN-γ in the HT29 cell line.

Analysis of Fas expression in peripheral blood samples

Venous blood (3 ml) was collected into K_3EDTA tubes prior to IFN-γ administration and at 48 h after the first IFN-γ dose; whole blood aliquots (100 μl) were stained according to vendor recommendations and immediately analyzed by FACS (Becton Dickinson FACS Scan, San Jose, Calif.) [15]. From the lymphocyte fraction, 5000 cells were collected and analyzed with CellQuest software. Anti-CD45, a general leukocyte marker, was used to gate out remaining red blood cells and debris and to monitor the quality of staining. Isotype antibodies with the appropriate fluorescent dyes were used as negative controls. To analyze the relative level of cell surface Fas in granulocytes, the following directly labeled monoclonal antibody (mAb) (Becton Dickinson) combinations in triple staining were applied using anti-CD45, anti-Fas, and one of anti-CD3-FITC, anti-CD4-FITC, anti-CD8-FITC, anti-CD19-FITC, anti-CD56-FITC (IgG2b), anti-CD14-FITC (IgG2b), or anti-CD15-FITC (IgM). Throughout the study the same lot (M044107) of anti-Fas (CD95) mAb (clone DX2) labeled with phycoerythrin (PE) (Pharmingen #33455X) was used. At each measurement the PE staining was calibrated with Quantibright (Becton Dickinson) beads. Mean fluorescence intensity was converted to number of PE molecules using Quantiquest software, and relative changes in the cell surface PE numbers were determined.

Fas upregulation by IFN-γ exposure in HT29 cells

We conducted a series of studies to assess the pharmacodynamic effect of IFN-γ on tumor cells (i.e., HT-29) at IFN-γ exposures similar to those observed in patients. Fas upregulation in HT29 cells was used as our pharmacodynamic endpoint. Cell surface Fas expression was determined in HT29 human colon carcinoma cells by FACS analysis with a phycoerythrin-conjugated DX2 anti-Fas mAb (Pharmingen, San Diego, Calif.) using standard procedures. We used a 6.5-h exposure to IFN-γ concentrations (ranging from 0 to 10 U/ml) and measured the upregulation of Fas in HT29 cells from 0 to 48 h [10]. We then repeated the same studies but with a 2-h exposure to 5-FU (38.5 μM, which is the 5-FU $AUC_{0 \to 2h}$ in this patient population [15]), LV (1 μM), and IFN-γ for 6.5 h (1–10 U/ml). This allowed us to assess the contribution of 5-FU/LV to Fas upregulation by IFN-γ.

Statistical methods

Fas upregulation was measured as the ratio of the Fas level obtained at a given time point (24 h, 48 h) to that observed at baseline. The Spearman correlation coefficient was used to examine the relationship between Fas upregulation and IFN-γ $AUC_{0 \to \infty}$. The Kruskal-Wallis test was used to explore differences in Fas upregulation with dose. Criteria for statistical significance were set a priori at $P < 0.05$. SAS (SAS Institute, Cary, N.C.) and StatXact (CYTEL Software Corporation, Cambridge, Mass.) were used for statistical analysis. No adjustments were made for multiple comparisons.

Results

Patients

Patient-specific demographic and disease-specific characteristics are summarized in Table 1. Our patient population represented a diverse group of diseases and extent and type of prior therapy. However, we were unable to identify a relationship between IFN-γ disposition and diagnosis or prior therapy. Similarly we were unable to find a statistically, clinically significant relationship between measures of hepatic or renal function (i.e., AST, ALT, alkaline phosphatase, and serum creatinine) and IFN-γ apparent systemic clearance.

Table 1 Summary of patient-specific characteristics

Sex (*n*)	
Male	10
Female	9
Diagnosis (*n*)	
Colorectal cancer	16
Gastric cancer	1
Pancreatic cancer	2
Race (*n*)	
African-American	5
Caucasian	14
Age (years)	
Median	64.5
Range	40.5–80.4
BSA (m^2)	
Median	1.99
Range	1.57– 2.29
Weight (lbs)	
Median	183
Range	119–267
Number of prior courses of chemotherapy	
Median	1
Range	1–4

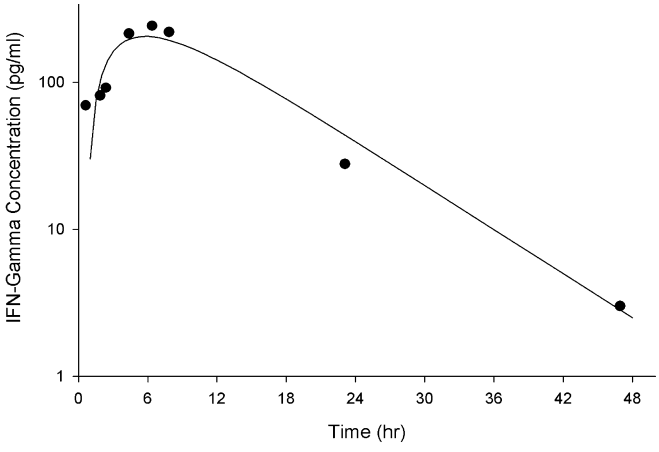

Fig. 1 Representative IFN-γ plasma concentration-time plot from one patient at the 100 $\mu g/m^2$ dosage. Individual data points and the best-fit line of the data are shown

Pharmacokinetic modeling

In order to model the disposition of s.c. IFN-γ in this patient population, we initially fitted a model of first-order absorption with linear elimination to the concentration data for the 13 patients for whom complete data sets were available. However, this model was unable to achieve convergence on the estimates of pharmacokinetic parameters. Because an apparent delay was observed in the appearance of IFN-γ in plasma after s.c. administration, we next incorporated a lag phase independent of dose and clearance into the model. With this approach, the model was able to achieve convergence on the estimates of pharmacokinetic parameters.

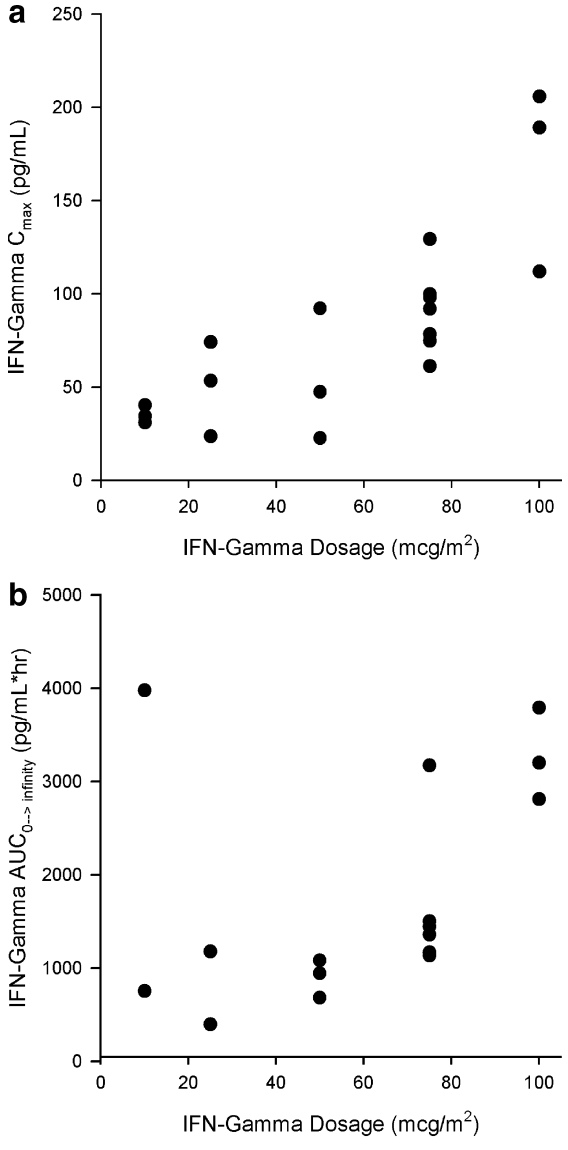

Fig. 2a, b Relationship between IFN-γ dosage and plasma C_{max} (**a**) and $AUC_{0 \rightarrow \infty}$ (**b**)

Depicted in Fig. 1 are the IFN-γ plasma concentration-time data with the best-fit line from a representative patient receiving 100 $\mu g/m^2$ IFN-γ. The model consisting of linear absorption with a delay and monophasic elimination after s.c. administration fitted the data. In addition, both C_{max} and $AUC_{0 \rightarrow \infty}$ increased with increasing dosage (Fig. 2).

Interpatient variability of pharmacokinetic parameters

The median (range) apparent systemic clearance was 46 l/m^2 per hour (2.5–93 l/m^2 per hour), which resulted in an approximately 37-fold range in clearance. Furthermore, there was 27-fold variability in $T_{1/2}$ with a median (range) of 6.8 h (2.8–75.8 h) and 12-fold variability in K_a with a median (range) of 0.26 h^{-1}

Table 2 Summary of pharmacokinetic model parameters ($n = 19$)

Parameter	Mean ± SD	Median
K_e (h^{-1})	0.12 ± .07	0.10
V/F (l/m^2)	361 ± 130	296
K_a (h^{-1})	0.29 ± 0.18	0.26
T_{lag} (h)	0.81 ± 0.30	0.71
T_{max} (h)	7.5 ± 3.3	6.5
CL/F ($l/m^2/h$)	42 ± 24	46
$T_{1/2}$ (h)	11 ± 16	6.8

Table 3 Summary of IFN-γ $AUC_{0\to\infty}$ and C_{max} at each dosage level. Values are medians (range)

IFN-γ dosage ($\mu g/m^2$)	$AUC_{0\to\infty}$ (pg/ml·h)	C_{max} (pg/ml)
10 ($n = 3$)	2365 (752–3977)	34.5 (31.1–40.4)
25 ($n = 3$)	788 (398–1177)	38.5 (23.7–53.4)
50 ($n = 3$)	942 (682–1083)	47.4 (22.6–92.2)
75 ($n = 7$)	1402 (1135–3172)	95.0 (74.8–129.4)
100 ($n = 3$)	3199 (2810–3793)	189.2 (111.9–205.8)

(0.064–0.78 h^{-1}). The range in T_{max} and T_{lag} was 3.5–20 h and 2.8–7.6 h, respectively, thus resulting in less interpatient variability than the other pharmacokinetic parameters. The mean ± SD and median are summarized in Table 2 for each pharmacokinetic model parameter.

For each dosage of IFN-γ the summary values for $AUC_{0\to\infty}$ and C_{max} are listed in Table 3. The IFN-γ AUC from zero to the last sample time point at 48 h accounted for greater than 90% of the $AUC_{0\to\infty}$ in all pharmacokinetic studies except in one patient at the 10 $\mu g/m^2$ dosage. Within each IFN-γ dosage level, we observed up to a 5.3- and 4-fold range in $AUC_{0\to\infty}$ and C_{max}, respectively.

Limited sampling model

To optimize timing and number of blood samples required to monitor IFN-γ disposition in future phase II studies, we used data from this pharmacokinetic study to create a LSM. From our analysis we determined that the optimal LSM for IFN-γ consists of four blood samples obtained at 4.5, 6.5, 28, and 30 h after IFN-γ administration. Since the actual samples were not drawn at these optimal sampling times, we used the model-estimated concentrations. Accuracy and bias were measured relative to the K_e and V calculated using all eight plasma samples. The median (quartile) accuracies for K_e and V were 12% (6–16%) and 13% (6–14%), respectively. The median (quartile) biases for K_e and V were −5% (−16–11%) and 4% (−12–14%), respectively. Both parameters were accurate ($P > 0.1$) and unbiased ($P > 0.25$).

Fas expression in peripheral blood samples

Based upon results from preclinical studies in human colon carcinoma cell lines that demonstrated sustained elevation of Fas expression for up to 48 h after IFN-γ exposure [18], IFN-γ was administered every other day. Thus we evaluated the expression of Fas in PBMCs 24 and 48 h after the first IFN-γ dose, compared to pretreatment levels of Fas expression. IFN-γ dosage was significantly associated with mean Fas upregulation only in the $CD4^+$ compartment at 24 h ($P = 0.024$) and the $CD56^+$ compartment at 48 h ($P = 0.044$). However, IFN-γ $AUC_{0\to\infty}$ was significantly associated with mean Fas upregulation in lymphocytes, and $CD15^+$, $CD3^+$, $CD4^+$, $CD8^+$, $CD56^+$ and $CD14^+$ cells at 24 h ($0.001 \le P \le 0.045$). All correlations were positive ($0.59 \le \text{rho} \le 0.83$), implying that higher measures of Fas upregulation were associated with larger values of $AUC_{0\to\infty}$. Also, IFN-γ $AUC_{0\to\infty}$ was significantly associated ($P \le 0.05$) with mean Fas upregulation in $CD15^+$ cells at 48 h (Fig. 3). Similarly, the time IFN-γ plasma concentrations remained above 33.3 pg/ml was significantly associated with mean Fas upregulation at 24 h in lymphocytes, and $CD15^+$, $CD4^+$ and $CD19^+$ compartments ($0.009 \le P \le 0.037$). All correlations were positive ($0.63 \le \text{rho} \le 0.74$), implying that higher levels of Fas upregulation were associated with longer durations of plasma concentrations of IFN-γ above a threshold value.

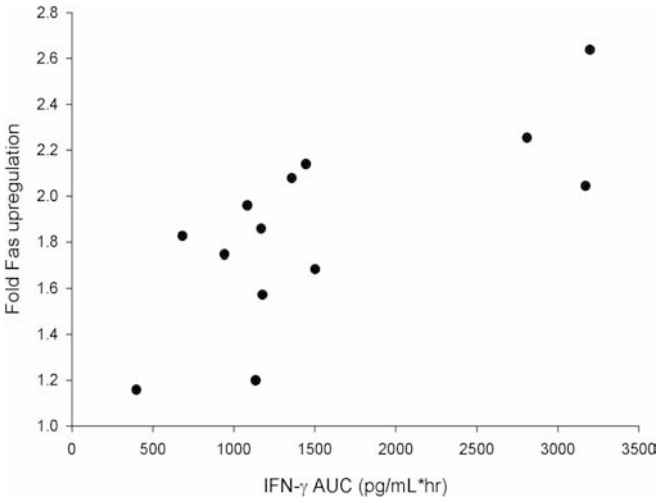

Fig. 3 Relationship between IFN-γ plasma $AUC_{0\to\infty}$ and Fas upregulation in $CD15^+$ cells at 48 h

Fas upregulation by IFN-γ exposure in HT29 cells

To enhance the design of in vitro studies in a human colon carcinoma cell line at clinically relevant IFN-γ exposures, we determined the median duration of IFN-γ plasma concentrations above 1, 3, and 5 U/ml for the MTD of IFN-γ in this combination with 5-FU/LV [15]. Figure 4 demonstrates the dose-dependent upregulation of Fas in the HT29 colon carcinoma cell line, using concentrations and durations of exposure determined to be clinically achievable in our patient population. After a 6.5-h exposure to IFN-γ alone (i.e., the median duration IFN-γ plasma levels remained above 5 U/ml in patients

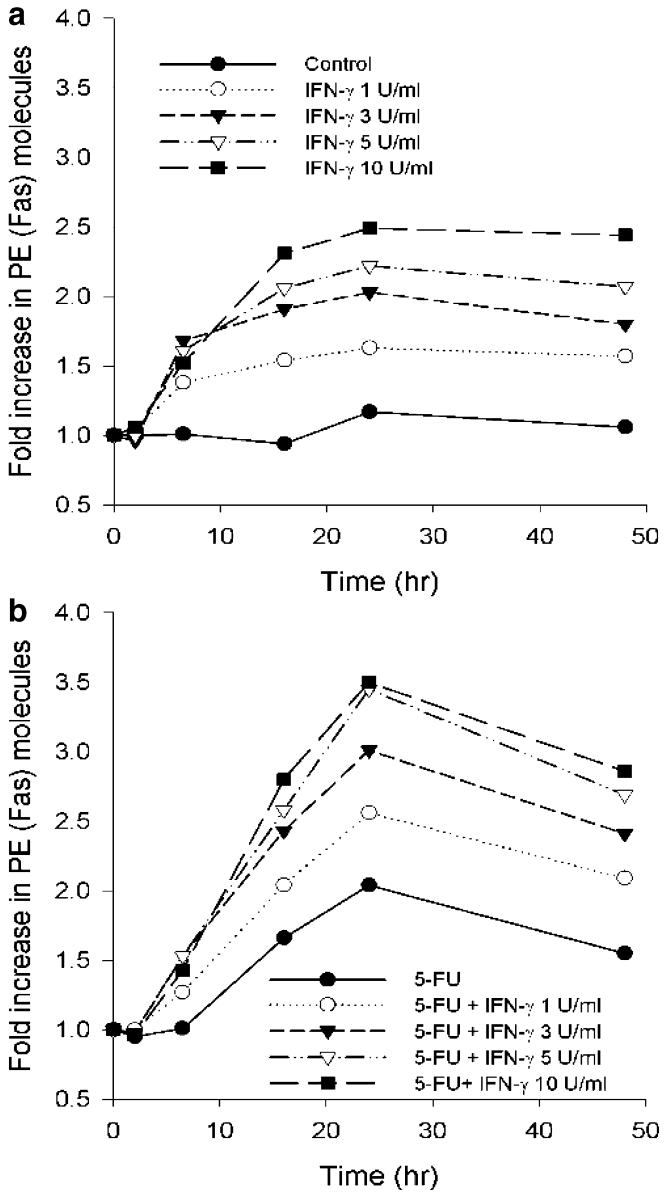

Fig. 4a, b Fas upregulation in HT29 cells (**a**) exposed to IFN-γ (0 to 10 U/ml) for 6.5 h, measured from 0 to 48 h, and (**b**) exposed to 5-FU (38.5 μM) and LV (1 μM) for 2 h, and IFN-γ (0 to 10 U/ml) for 6.5 h, measured from 0 to 48 h. Data are representative of three independent assays

receiving 100 $\mu g/m^2$) [15], the upregulation of Fas continued to increase until 16 h to reach a plateau that was maintained for at least 48 h. By 24 h after an exposure to 10 U/ml (333.3 pg/ml) of IFN-γ, Fas expression had increased 2.5-fold from baseline. Moreover, as depicted in Fig. 4, IFN-γ further induced the upregulation of Fas in HT29 cells exposed to 38.5 μM 5-FU combined with 1 μM LV, which is a pharmacologically achievable concentration in this patient population. In the cells exposed to IFN-γ, 5-FU, and LV, Fas upregulation continued to increase until it reached an apparent peak at 24 h. Both 5 and 10 U/ml (166.5 and 333.3 pg/ml) IFN-γ in combination with 5-FU/LV led to a 3.5-fold increase in Fas expression at 24 h. All of the concentrations of IFN-γ tested led to maintenance of a twofold or greater increase in Fas expression for at least 48 h.

Discussion

This is the first comprehensive pharmacokinetic and pharmacodynamic analysis of IFN-γ in patients with advanced gastrointestinal malignancies. In this study we evaluated the disposition of s.c. IFN-γ over a wide dosage range, and observed a lag of approximately 1 h in the first order, linear absorption. Furthermore, we showed that the elimination was fitted by a linear, one-compartment model. Moreover, the results of our pharmacodynamic analysis showed that in vivo the predicted pharmacological effect of IFN-γ occurs in PBMC (i.e., IFN-γ upregulates Fas death receptor expression in granulocytes and $CD56^+$ cells). Furthermore, at pharmacologically attainable IFN-γ concentrations Fas is upregulated in the HT29 human colon carcinoma cell line.

Data from our study showed that IFN-γ displayed a linear absorption with a lag phase and monophasic elimination. Other pharmacokinetic models of absorption and elimination were evaluated and proved statistically inferior to the model presented (data not shown). As has been described for other drugs administered s.c. [11, 19], a slight (e.g., <1 h) delay occurs before drug concentrations are measurable in the plasma. Without this delay incorporated into the model we were unable to achieve convergence on the pharmacokinetic parameter estimates. Since we have been unable to find other publications in which investigators performed compartmental analysis of their IFN-γ concentration-time data we cannot compare this aspect of our analysis. The monophasic elimination of IFN-γ that we observed is consistent with published reports of IFN-γ pharmacokinetics [5, 17].

Only limited published data are available regarding IFN-γ pharmacokinetics in adults. Thompson and colleagues conducted a dose escalation study of daily s.c. IFN-γ (0.5 to 8 MU/m^2 per day or 16.7 to 133.3 $\mu g/m^2$) as a single agent in patients with renal cell carcinoma, malignant melanoma, lymphoma, mesothelioma, breast carcinoma, liposarcoma, and multiple myeloma [17]. They reported an absorption half-life of 9–20 h, a T_{max} between 6.7 and 13 h, and serum elimination half-life of 2–3 h. These investigators also reported areas under the serum concentration time curve (AUC) for IFN-γ ranging from 25,650 to 287,000 pg/ml·h, and serum C_{max} ranging from 1.9 to 17.1 ng/ml, values that are more than 25-fold greater than we have observed in plasma. These differences might be explained by at least thee factors: drug formulation, method of quantification, and biological matrix. We studied a recombinant IFN-γ consisting of 140 amino acids rather than the 146 amino acid cytokine used by Thompson and colleagues. In order to determine the concentration of IFN-γ

present in the biological matrix we used ELISA, whereas Thompson and colleagues used a radioimmunoassay. Lastly, we determined the disposition of IFN-γ in plasma, and Thompson et al. characterized the disposition of IFN-γ in serum.

In another study of IFN-γ pharmacokinetics in adults, Digel and colleagues administered s.c. IFN-γ as a single agent to patients with renal cell carcinoma, lymphoma, colon carcinoma, liver cell carcinoma, neurosarcoma, osteosarcoma, and pancreatic sarcoma [5]. They reported a mean C_{max} of 4.6 ng/ml for a dose of 100 $\mu g/m^2$. Over the entire dosage range of 100 to 500 $\mu g/m^2$, the mean T_{max} was 6.4 h [5].

The plasma IFN-γ concentration-time data from the present study were well-described by a pharmacokinetic model characterized by a lag-phase and monoexponential elimination. The pharmacokinetic parameter estimates for the model were for most patients precise (e.g., CV% <25); however, as with other anticancer drugs we have studied and as has been reported with IFN-γ, wide interpatient variability characterized many of the pharmacokinetic parameter estimates. The IFN-γ $AUC_{0 \rightarrow 48h}$ accounted for $>90\%$ of the $AUC_{0 \rightarrow \infty}$ in all pharmacokinetic studies except in one patient at the 10 $\mu g/m^2$ dosage, who had received three prior courses of treatment for colon cancer. In this patient the IFN-γ plasma concentrations remained at a plateau through the end of the sampling window; thus, we were not able to characterize the IFN-γ elimination phase. Since we were unable to identify any statistically significant covariates between IFN-γ pharmacokinetic parameters and measures of hepatic or renal function, it is unlikely that we could account for this single observation.

In addition to characterizing the IFN-γ plasma pharmacokinetics, we developed a LSM consisting of the optimal times when IFN-γ levels should be drawn that could be used in future studies of IFN-γ pharmacokinetics. Limiting the sampling to four samples (compared with the eight samples from patients in this study) may reduce the inconvenience to patients, their families, and clinic staff. Furthermore, the reduction in samples obtained for clinical pharmacology studies will reduce the costs associated with assays. Although we recognize that this limited sampling scheme was developed from a small population ($n=19$), this model was reasonably accurate and unbiased when evaluated in our study population. However, this LSM should serve as a first approximation and will require validation in further patient populations.

The clinical relevance of the pharmacokinetic data from this study will be apparent upon completion of phase II studies of IFN-γ with 5-FU/LV in patients with colorectal carcinoma powered sufficiently to determine efficacy of this novel combination. Although biochemical modulation of 5-FU with LV has been the standard of pharmacotherapy for this disease, the FDA has recently approved a regimen including irinotecan, 5-FU, and LV as front-line treatment [13]. However, excessive toxicity to this regimen has caused a re-evaluation of this approach [12, 14], and further advances in pharmacologically rational therapy are needed. Recently we reported the use of IFN-γ with 5-FU and LV in adults with gastrointestinal malignancies and observed a promising response rate. This regimen is now under evaluation in a phase II clinical trial.

Although this pharmacokinetic study was not designed to assess efficacy, we were able to characterize the pharmacological effect of adding IFN-γ to 5-FU/LV in a surrogate compartment and an in vitro model system of colorectal carcinoma. We evaluated IFN-γ pharmacodynamics in patients and in HT29 colon carcinoma cells, and found that IFN-γ exposures greater than or equal to 33.3 pg/ml were sufficient to upregulate Fas expression in HT29 cells in vitro and in human $CD15^+$ cells in vivo. This upregulation occurred rapidly (i.e., within 6 h), and persisted for approximately 24 h at 75 $\mu g/m^2$ of IFN-γ, which was determined in a phase I study as the MTD [15].

Furthermore, we conducted a statistical analysis to characterize the pharmacodynamic association between IFN-γ $AUC_{0 \rightarrow \infty}$ and Fas upregulation in PBMC compartments. The paucity of significant associations between IFN-γ dosage and Fas upregulation in PBMC subcompartments is likely due to the large variability in IFN-γ pharmacokinetics and systemic exposure at a given dosage. However, we demonstrated a significant association between IFN-γ $AUC_{0 \rightarrow \infty}$ and Fas upregulation in $CD15^+$ cells at 24 h, but this association was stronger in $CD4^+$ and $CD56^+$ cells. There was a preponderance of significant associations between IFN-γ systemic exposure and Fas upregulation in several PBMC subcompartments. Thus, it may be possible to predict biological response to IFN-γ by measuring IFN-γ plasma levels and determining an individual's IFN-γ systemic exposure. Similarly, the association between Fas upregulation and the time IFN-γ plasma concentrations remain above 33.3 pg/ml (1 U/ml) is significant in both the $CD15^+$ and $CD4^+$ compartments, but the association is stronger in the $CD4^+$ compartment. The strong correlation between IFN-γ pharmacokinetics, especially $AUC_{0 \rightarrow \infty}$, with measures of biological response (e.g., Fas upregulation in specific PBMC compartments) may potentially be useful in minimizing variability in response to IFN-γ by minimizing variability in IFN-γ systemic exposure. But first the correlation between toxicity or response and IFN-γ pharmacokinetics or pharmacodynamics must be established in phase II studies. Moreover, further studies will be required to determine which PBMC compartment is the most robust surrogate marker of Fas upregulation by IFN-γ, but these results suggest that further prospective validation of the $CD56^+$ and the $CD15^+$ compartments is needed.

Not only did IFN-γ upregulate Fas expression in the PBMC compartment of this patient population, but also in vitro in the HT29 colon carcinoma cell line IFN-γ upregulated Fas expression at pharmacologically attainable concentrations. Under these conditions in the HT29 cell line, the time course of Fas upregulation with

a peak at 24 h and a modest decline at 48 h very closely approximated the time course of Fas upregulation that we observed in the $CD15^+$ cells in patients treated with 5-FU/LV/IFN-γ [15]. Moreover, 5-FU/LV in the absence of IFN-γ also upregulated Fas expression in vitro, but this effect was potentiated by clinically achievable IFN-γ concentrations and durations of exposure. The sustained Fas upregulation in vitro for at least 48 h provides further rationale for every-other-day dosing of s.c. IFN-γ when the pharmacological target is potentiation of the antitumor activity of 5-FU and LV. Moreover, we observed that Fas expression increased with increasing IFN-γ dosages. Although the highest IFN-γ dosage evaluated in this study was above the MTD determined in the recently published phase I study, higher IFN-γ dosages might be tolerated if administered on alternative schedules (e.g., twice weekly vs thrice weekly), with different 5-FU/LV regimens (e.g., de Gramont [4] vs Mayo Regimen [6]), or in newly diagnosed patients.

Based upon the promising results of the recently published clinical trial [15] with IFN-γ administered in combination with 5FU/LV for gastrointestinal malignancies, further studies of this combination in phase II are planned. The results of the present pharmacology study will enhance the ability of the clinician to dose IFN-γ in these phase II clinical trials. Moreover, the LSM developed in our study will enhance the conduct of further pharmacokinetic and pharmacodynamic studies of IFN-γ in this context.

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