Pharmacokinetic and pharmacodynamic comparison of two doses of calcium folinate combined with continuous fluorouracil infusion in patients with advanced colorectal cancer

• Sigrid Stremetzne, Michael Streit, Ernst-Dietrich Kreuser, Walter Schunack and Ulrich Jaehde

Pharm World Sci 1999;21(4): 184-189.

Sigrid Stremetzne, Walter Schunack, Ulrich Jaehde (correspondence): Institute of Pharmacy I, Department of Clinical Pharmacy, Freie Universität Berlin, Kelchstr. 31, D-12169 Berlin, Germany
Michael Streit, Ernst-Dietrich Kreuser: Department of Hematology and Oncology, University Hospital Benjamin Franklin, Freie Universität Berlin, Hindenburgdamm 30, D-12203 Berlin, Germany

Keywords
Chemotherapy
Colorectal cancer
Fluorouracil
Folinate
Leucovorin
Pharmacokinetics
Toxicity

Abstract
The optimum dose of calcium folinate (leucovorin) as modulator of fluorouracil has not been defined yet. We conducted a randomized trial to compare the pharmacokinetics/ pharmacodynamics of two doses of calcium folinate. 16 patients with advanced colorectal cancer were treated with 650 mg/m^2/d fluorouracil as 5 day continuous infusion and randomized to receive either 20 mg/m^2 or 100 mg/m^2 calcium folinate as short infusion twice daily. The two diastereoisomers of calcium folinate were analyzed separately by chiral HPLC to account for differences in their pharmacokinetics. The pharmacokinetics of fluorouracil was not affected by folinate dosing. Total clearance of the active (6S)-diastereoisomer was found to be lower after the higher dose of folinate which can be explained by nonlinear metabolism. The incidence of treatment-induced mucositis significantly increased with (6S)-folinate exposure, whereas fluorouracil exposure was not related to this type of toxicity. In conclusion, exposure to folinate is more important for toxicity in this regimen than fluorouracil pharmacokinetics. Therefore, monitoring of fluorouracil plasma levels is not useful in this combination. Our results show that folinate dose should be carefully selected. Lower doses of folinate might be preferred because of less toxicity compared to higher doses.

Accepted November 1998

Introduction

For more than 30 years fluorouracil has been used in the treatment of gastrointestinal carcinomas. The cytotoxic activity of fluorouracil is enhanced by coadministration of calcium folinate (leucovorin) due to more pronounced inhibition of thymidilate synthase compared to fluorouracil alone. This effect is explained by increased intracellular concentrations of 5,10-methylene-tetrahydrofolate which forms a stable ternary complex with 5-fluoro-desoxyuridine monophosphate (FdUMP), a metabolite of fluorouracil, and thymidilate synthase [1]. The combination of fluorouracil and calcium folinate represents one of the current standard treatments of metastatic colorectal cancer. Calcium folinate was shown to be superior to other biomodulators [2]. Response rates range from 10 to 60% [3].

Although many different regimens of fluorouracil and calcium folinate have been used the optimum folinate dosage is still a controversial issue. The folinates doses used varied from 20 mg/m^2 weekly [4] to 500 mg/m^2 daily [5]. On one hand, inhibition of thymidilate synthase was found to be more pronounced using higher doses of calcium folinate [6]. In a large phase III trial there was a trend toward longer survival when high-dose folinate was used compared to lower doses [7]. On the other hand, some authors favor low doses because of less treatment-related toxicity [8,9].

Dosage recommendations should be based on clinical trials as well as sound pharmacokinetic and pharmacodynamic investigations. Commercially available calcium folinate is a mixture of equal amounts of two diastereoisomers. (6S)-folinate is converted to the active metabolite 5,10-methylene-tetrahydrofolate, whereas the inactive (6R)-folinate is mainly excreted unchanged by the kidneys. Consequently, both diastereoisomers differ considerably in their pharmacokinetics. (6S)-folinate exhibits a shorter half-life compared to the (6R)-diastereoisomer [10 11 12]. Recent investigations focussed on administration of pure (6S)-folinate. These trials indicated that (6S)-folinate pharmacokinetics does not exhibit major differences when given separately or as diastereoisomeric mixture [13 14].

Although the pharmacokinetics of fluorouracil and the two diasteromers of calcium folinate was already described in other clinical trials [10 11 12] there is no randomized pharmacokinetic and pharmacodynamic comparison of two different dosages of calcium folinate in combination with fluorouracil. In order to clarify which folinate dose should be preferred we conducted this randomized trial in patients with advanced colorectal cancer using continuous infusion of fluorouracil combined with pulsed folinate. This regimen has been shown to be effective as second-line therapy in advanced colorectal cancer with acceptable toxicity preserving the quality of life during chemotherapy [15]. Our study design allowed to reveal a potential effect of different folinate doses on fluorouracil pharmacokinetics, to investigate a potential time- and dose-dependence of (6S)- and (6R)-folinate kinetics, and to characterize relationships between pharmacokinetics and pharmacodynamics of fluorouracil/folinate.

Methods

Patients

16 patients with advanced colorectal cancer were investigated. Patients with histologically confirmed metastatic unresectable tumor, estimated life expectancy of more than three months and at least one

measurable tumor parameter were included in this trial. Moreover, the following inclusion criteria were defined: Karnofsky performance status ≥ 50%, sufficient blood cell counts (WBC > 4000/mm^3, platelet count > 100,000/mm^3), liver function (bilirubin level < 1.75 mg/dl), and renal function (serum creatinine level < 1.5 mg/dl). Patients with cardiac infarction up to six months before start of treatment or pregnant women were excluded from the study.

Four women and twelve men with median age of 63 years (range: 49-76 years), median weight of 72 kg (range: 55-105 kg) and median height of 168 cm (range: 155-190 cm) were included in this trial. Estimated body surface area resulted in a median value of 1.8 m^2 ranging from 1.5 to 2.2 m^2. All patients gave written informed consent to participate in the study. The study protocol was approved by the local ethics committee.

Study design

Fluorouracil was administered as 5 day continuous infusion in a dosage of 650 mg/m^2/d. Patients were randomized to receive either 20 mg/m^2 or 100 mg/m^2 calcium folinate (Rescuvolin®) as diastereoisomeric mixture. Calcium folinate was given as short infusion over 15 minutes every 12 hours during fluorouracil infusion. In total, each patient received ten short infusions of calcium folinate.

On the first treatment day and on day 3, 4 or 5 when steady-state of both folinate diastereoisomers was reached serial blood samples of 4 ml blood were drawn before start of infusion, at the end of infusion, 20, 40, 60, 90 minutes, and 2, 3, 4, 6, and 12 hours after the end of infusion of calcium folinate. The same sampling protocol was used on both days. Blood was collected in heparinized tubes and immediately mixed with 0.1 mg of ascorbic acid to avoid oxidation of calcium folinate. After 10 min centrifugation (3000 rpm) plasma aliquots were stored at -70 °C until analysis.

Drug analysis

Plasma samples were analyzed for fluorouracil by reversed phase ion-pair HPLC using an assay modified from Christophidis et al. [16] and Quebbeman et al. [17]. In brief, Spherisorb® S ODS 2.5 µm (Knauer GmbH, Berlin, Germany) was used as stationary phase. The mobile phase consisted of 0.05 M Na_2HPO_4 and 0.01 M tetrabutylammonium hydroxide adjusted to pH 6.2. Fluorouracil was detected by UV absorbance at 266 nm. The following extraction procedure was used: 50 µl of an aqueous solution of the internal standard bromouracil and 50 µl 1 M phosphate buffer (pH 4.8) were added to 200 µl plasma. Afterwards the sample was centrifuged with 3.0 ml of a mixture of diethylether and 1-propanol (84:16, V/V). The organic phase was separated and centrifuged with 200 µl 0.05 M phosphate buffer (pH 11) in order to extract fluorouracil and bromouracil. The aqueous phase was separated, dried using vacuum evaporation and dissolved in 200 µl purified water. 20 µl 0.07 M phosphoric acid was added to obtain a pH of 7.0, and 80 µl of this mixture was injected onto the column. The calibration curves were linear between 30 and 570 ng/ml. Within-day precision of the assay was assessed by 10fold injection of three different plasma samples spiked with a low, medium and high fluorouracil concentration. Coefficients of variation ranged from 0.4 to 3.2%. Mean between-day precision using biologically derived plasma samples was found to be 7.5%.

The diastereoisomers of calcium folinate were analyzed separately by chiral HPLC [18]. The stationary phase consisted of silica with covalently bound bovine serum albumin (Resolvosil®, Macherey-Nagel, Düren, Germany). The reference compounds were kindly supplied by Medac, Hamburg, Germany. 0.05 M Na_2HPO_4 adjusted to pH 5.2 was used as mobile phase. Under these chromatographic conditions (6S)-folinate had a shorter retention time compared to the (6R)-diastereoisomer. Detection was performed by UV absorbance at 290 nm. Before analysis, 500 µl plasma was deproteinized by addition of 1000 µl acetonitril and centrifuged. The supernatant was dried using vacuum evaporation and dissolved in 50 µl purified water. 20 µl of the solution was injected onto the column. The calibration curves of both folinate diastereoisomers were linear between 50 ng/ml and 20 µg/ml. Within-day precision assessed as described for fluorouracil ranged between 0.3 and 1.7% for (6S)-folinate and between 0.2 and 1.1% for (6R)-folinate, respectively. Mean between-day precision was found to be 2.6% for both diastereoisomers.

Pharmacokinetic analysis

Pharmacokinetic parameters of fluorouracil were calculated using non-compartmental methods. Since no circadiane fluctuations were observed between 2 p.m. and 10 p.m. (see results and discussion) steady-state concentrations were calculated as average plasma concentration during this time interval. Total clearance (CL) was calculated by dividing infusion rate by steady-state concentration (C_{ss}), area under the plasma-concentration curve (AUC) was obtained by the trapezoidal rule. The pharmacokinetic parameters AUC, CL, V_{ss} and terminal $t_{1/2}$ of the two folinate diastereoisomers were estimated using a one-compartment model for (6S)-folinate and a two-compartment model for (6R)-folinate. Curve fitting was performed by a weighted least-squares optimization procedure (SIPHAR/Win, Simed, Créteil, France). Peak plasma levels (C_{max}) were taken directly from the plasma level-time curve.

Pharmacodynamic analysis

For evaluation of response WHO criteria were used. In addition, "minor remission" (MR) was defined as a reduction of tumor parameters of less than 50% but at least 25% without hint of enlarged or newly detected metastases up to 4 weeks after treatment. Toxicity was graded according to NCI criteria.

Statistical analysis

Statistical differences in mean pharmacokinetic parameters were evaluated by either one-way or two-way ANOVA (folinate dose and day of treatment) followed by the Tukey test (Statgraphics, Statistical Graphics Corp., Rockville, USA). The Kolmogoroff-Smirnoff test was used to confirm normal distribution. The AUC values of patients with and without toxicity were compared using the Mann-Whitney test. All p values < 0.05 were considered to be statistically significant.

Results

Pharmacokinetics of fluorouracil

Figure 1 shows mean plasma levels of fluorouracil versus time of the day. All plasma levels under steady-state conditions were hourly summarized. Whereas there was a considerable variability, we did not observe any major circadiane fluctuation during daytime. Since concentrations around 11 a.m. tended to be lower compared to afternoon and evening we decided to calculate the steady-state concentration of fluorouracil as mean concentration between 2 p.m and 10 p.m. In one patient considerably lower fluorouracil levels (< 50 ng/ml) were observed without reasonable explanation. This patient was excluded from pharmacokinetic and statistical evaluations for fluorouracil. Steady-state concentrations, total plasma clearance and $AUC_{0\text{-}12\ h}$ for each day of treatment were calculated for all individual patients. Mean parameters are listed in Table 1 separately for the dose of calcium folinate and the day of treatment. No significant effect of folinate dose or the day of treatment on pharmacokinetics of fluorouracil was observed. Although CL of patients receiving the higher dose of calcium folinate seemed to be elevated the difference between this group and the others was not significant.

Pharmacokinetics of (6S)- and (6R)-folinate

As expected considerable pharmacokinetic differences were found between both diastereoisomers. (6S)-folinate exhibited significantly lower peak levels, a higher total plasma clearance and a shorter terminal half-life compared to the inactive (6R)-diastereoisomer.

Active (6S)-folinate showed an almost identical concentration-time profile on day 1 and day 3-5 with a monoexponential decline (Figure 2). As half-life of the active diastereoisomer is extremely short (15 to 21 minutes) plasma levels after administration of the lower dose reached the lower limit of quantification 1.2 hours after the end of infusion. After the higher dose of 100 mg/m^2 (6S)-folinate could be measured up to 2.3 hours after the end of folinate infusion. Comparing plasma levels after administration of the two different folinate dosages a faster decline was found after administration of 20 mg/m^2. Mean pharmacokinetic parameters of (6S)-folinate are listed separately for folinate dose and day of treatment (Table 2). Peak levels (C_{max}) and systemic exposure (AUC) of (6S)-folinate were found to increase overproportionally with dose. Patients receiving 20 mg/m^2 of folinate exhibited a shorter half-life and higher total clearance ($p<0.05$) compared to those with 100 mg/m^2. Volume of distribution (V_{ss}) did not differ significantly between doses indicating that dose-dependence of (6S)-folinate pharmacokinetics results from nonlinear elimination.

Plasma levels of the inactive (6R)-folinate declined biexponentially (Figure 3) with a terminal half-life between 6.6 and 8.6 h. Consequently, cumulation of the (6R)-diastereoisomer was observed during the 5 day treatment. As plasma levels of active (6S)-folinate did not differ between day 1 and days 3-5 the (6S/6R)-concentration ratio decreased continuously until (6R)-folinate levels reached steady-state. Concentration-time profiles of (6R)-folinate after the two administered doses were parallel. Table 3 summarizes the pharmacokinetic parameters calculated

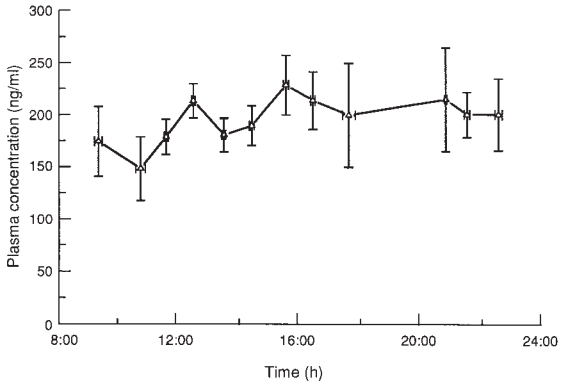

Figure 1

Mean plasma concentrations (± standard error of the mean) of fluorouracil (n=15; one patient with extremely low concentrations was excluded, see text) versus time of the day (plasma concentrations were hourly summarized) during continuous infusion.

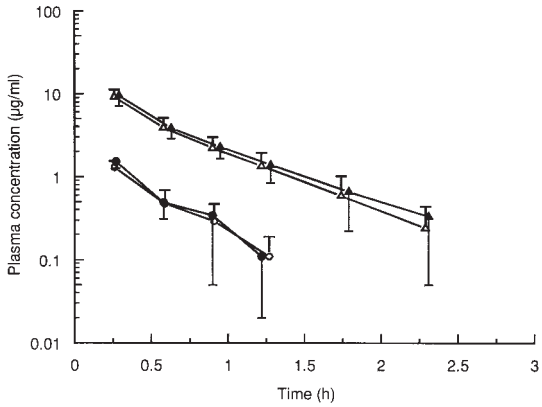

Figure 2

Mean plasma concentrations (± standard deviation) of calcium (6S)-folinate after i.v. administration of 20 mg/m^2 (• day 1, o days 3-5) and 100 mg/m^2 (▲ day 1, △ days 3-5).

Table 1 ***Pharmacokinetic parameters (mean ± standard deviation) of fluorouracil (n=8)***

Folinate dose (mg/m^2)	*Day of treatment*	*C_{ss}(ng/ml)*	*CL(ml/min)*	*$AUC_{0\text{-}12\ h}$(ng•h/ml)*
20	1	203 ± 75	4211 ± 1251	2426 ± 1050
20	3-5	190 ± 47	4498 ± 1522	2196 ± 617
100	1	165 ± 74*	5715 ± 1713*	1995 ± 850*
100	3-5	216 ± 71	4172 ± 1147	2716 ± 937

*n=7 (one patient was excluded because of extremely low plasma concentrations, see text)

for the inactive (6R)-diastereoisomer. In contrast to the active diastereoisomer, C_{max} and AUC proportionally increased with dose. Accordingly, no significant differences in total clearance, terminal half-life or volume of distribution between the two folinate doses and between the different treatment days were found. For this reason, linear pharmacokinetics of (6R)-folinate can be assumed over the dose range studied.

Relationships between pharmacokinetics and pharmacodynamics

Tumor response and treatment-related toxicity are summarized in Table 4. 14 out of 16 patients showed a stable disease, one patient had a progressive disease, and in one patient a minor remission (MR) was observed. The latter two patients did not show any outstanding pharmacokinetic characteristics compared to the others.

Dose-limiting toxicity in this regimen was mucositis and hand-foot syndrome. Half of the patients experienced treatment-induced mucositis (5 patients grade 2, 3 patients grade 3). Patients with mucositis had a slightly higher systemic exposure to fluorouracil compared to those with no mucositis (Figure 4). However, the difference in fluorouracil AUC (calculated as the sum of the AUC values during the two observed days) was not significant. In Figure 4 folinate dose is indicated for individual patients. It is evident that all patients receiving the higher dose experienced mucositis whereas only one patient with the low dose showed this toxicity suggesting that mucositis mainly depends on folinate exposure. In Figure 5 total AUC of (6S)-folinate on both observation days is plotted separately for patients with and without mucositis. Patients with mucositis clearly had a higher exposure to the active (6S)-diastereoisomer compared to patients without mucositis. Interestingly, the patient exhibiting the highest AUC of the low-dose group experienced mucositis. Thus, folinate exposure is clearly an important factor for the incidence of mucositis in fluorouracil/folinate regimens.

Four patients developed a hand-foot syndrome which is characteristic for fluorouracil-containing regimens [19]. Palms and soles were symmetrically swollen and showed marked erythema. On day 1 mean

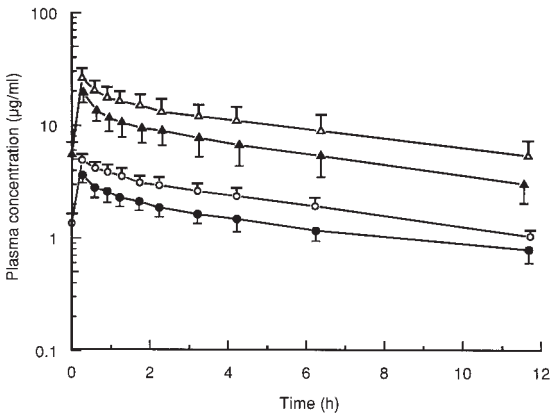

Figure 3

Mean plasma concentrations (± standard deviation) of calcium (6R)-folinate after i.v. administration of 20 mg/m² (• day 1, o days 3-5) and 100 mg/m² (▲ day 1, Δ days 3-5).

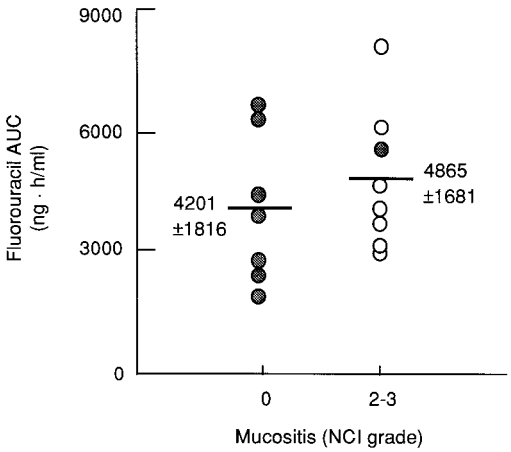

Figure 4

Relationship between fluorouracil AUC (mean ±standard deviation) and treatment-induced mucositis (• 20 mg/m² folinate, o 100 mg/m² folinate).

Table 2 ***Pharmacokinetic parameters (mean ± standard deviation) of calcium (6S)-folinate (n = 8)***

Folinate dose (mg/m²)	*Day of treatment*	*C_{max} (µg/ml)*	*AUC (µg•h/ml)*	*CL (ml/min)*	*$t_{1/2}$ (h)*	*V_{ss} (l)*
20	1	1.51 ± 0.24	0.79 ± 0.23	408 ± 141	0.25 ± 0.08	8.14 ± 1.63
20	3-5	1.30 ± 0.25	0.78 ± 0.25	427 ± 180	0.31 ± 0.13	10.02 ± 2.66
100	1	9.44 ± 2.35	6.10 ± 1.27	255 ± 82	0.35 ± 0.05	7.58 ± 2.13
100	3-5	9.15 ± 2.07	5.77 ± 1.63	306 ± 201	0.34 ± 0.08	8.33 ± 3.52

Table 3 ***Pharmacokinetic parameters (mean ± standard deviation) of calcium (6R)-folinate (n = 8)***

Folinate dose (mg/m²)	*Day of treatment*	*C_{max} (µg/ml)*	*AUC (µg•h/ml)*	*CL (ml/min)*	*$t_{1/2\,\alpha}$ (h)*	*$t_{1/2\,\beta}$ (h)*	*V_{ss} (l)*
20	1	3.61 ± 0.51	25.75 ± 6.70	12.39 ± 3.79	0.50 ± 0.14	8.20 ± 3.18	7.90 ± 2.52
20	3-5	4.92 ± 0.63	26.51 ± 3.89	11.58 ± 2.47	0.64 ± 0.32	8.60 ± 1.20	7.93 ± 1.35
100	1	19.40 ± 3.58	104.83 ± 28.32	15.08 ± 4.74	0.28 ± 0.15	6.57 ± 1.63	8.16 ± 3.58
100	3-5	26.07 ± 5.85	122.01 ± 33.12	13.10 ± 4.68	0.37 ± 0.15	7.37 ± 1.68	7.99 ± 4.09

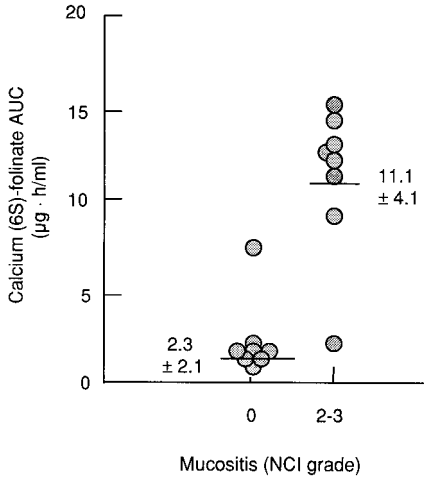

Figure 5
Relationship between calcium (6S)-folinate AUC (mean ± standard deviation) and treatment-induced mucositis.

Table 4 *Response rates and toxicity*

Patient	*Treatment cycle*	*Response rate*	*Toxicity*
1	1	SD	none
2	1	SD	none
3	1	SD	mucositis grade 2 hand-foot syndrome grade 3
4	1	SD	none
5	5	PD	mucositis grade 2
6	3	SD	mucositis grade 2 hand-foot syndrome grade 3
7	1	SD	mucositis grade 2
8	3	SD	hand-foot syndrome grade 2
9	1	SD	mucositis grade 3
10	2	SD	hand-foot syndrome grade 1
11	1	SD	mucositis grade 2
12	3	MR	none
13	1	SD	diarrhea grade 2
14	3	SD	mucositis grade 3
15	2	SD	nausea grade 2
16	1	SD	mucositis grade 3

fluorouracil plasma levels of patients with hand-foot syndrome were slightly higher compared to those without hand-foot syndrome but on days 3-5 plasma levels were comparable. In addition, folinate kinetics was not conspicuous in these patients. Thus, our data do not suggest any relationship between drug exposure and the development of a hand-foot syndrome.

Discussion

This trial investigated the pharmacokinetics and pharmacodynamics of a combined treatment with fluorouracil and calcium folinate. Calculated pharmacokinetic parameters for fluorouracil, (6S)- and (6R)-folinate were comparable to those reported by other authors [10,11,12].

Our data clearly show that repeated administration of different doses of calcium folinate has no influence on fluorouracil pharmacokinetics. After continuous infusion circadiane fluctuations of fluorouracil kinetics were reported [20,21]. Metzger et al. [21] investigated a similar dosage to the one used in this study and reported maximum concentrations of fluorouracil around 4 a.m. and minimum concentrations around 1 p.m. Since in our study no samples were drawn at night, chronopharmacokinetics was less evident. The lowest plasma levels were observed around 11 a.m.

The considerable differences in the pharmacokinetics of the two diastereoisomers of folinate underline the importance of separate analysis. Active (6S)-calcium folinate exhibited a higher total clearance and a shorter elimination half-life compared to the inactive diastereoisomer. After repeated dosing only the inactive diastereoisomer undergoes cumulation. Since (6S)-folinate kinetics does not alter during the 5 day treatment, it seems unlikely that the large excess of (6R)-folinate inhibits intracellular uptake and efficacy of (6S)-folinate which is in accordance with results of Bertrand et al [22]. Nonlinear elimination of (6S)-folinate was already suggested by Newman et al. [23] comparing three dose levels consecutively in a dose escalation study. For the first time we describe nonlinear elimination of (6S)-folinate using a randomized comparison of two doses. The lower clearance that we found after the high folinate dose might be explained by saturable metabolism. This interpretation is supported by the fact that the (6R)-diastereoisomer which is not metabolized does not show dose-dependent kinetics. Schilsky and Ratain [24] found a considerably lower total clearance and longer half-life of (6S)-folinate after administration of a much larger dose (1000 mg) of folinate to 5 healthy volunteers compared to our data. On the other hand, clearance and half-life of (6R)-folinate were similar to those reported in our study.

Some adverse drug reactions associated with fluorouracil chemotherapy depend on the administration mode of the drug. When fluorouracil is given as bolus injection, hematotoxicity is dose-limiting, whereas continuous infusion is mainly associated with mucositis [25, 26]. Our data do not indicate a significant relationship between fluorouracil kinetics and the incidence of toxicity. This is in contrast to Thyss et al. [27] who established a relationship between individual fluorouracil exposure and treatment-induced toxicity in head and neck cancer patients receiving fluorouracil as continuous infusion over 5 days without folinate coadministration. Although our patients with mucositis exhibited slightly higher fluorouracil AUC values, the difference was not significant. However, our data clearly demonstrate the importance of folinate dose and exposure for toxicity. Patients who received 100 mg/m^2 of calcium folinate had a significantly increased risk of treatment-related mucositis. Routine monitoring of fluorouracil plasma levels might hence be of less value for minimizing toxicity when the drug is combined with folinate. A careful selection of the appropriate folinate dose seems to be of more relevance. Moreover, the higher folinate dose apparently did not improve treatment outcome in our patients. This observation is in accordance with a

recent multicenter trial of Jäger et al. [8] who compared 20 mg/m^2 and 500 mg/m^2 calcium folinate combined with 500 mg/m^2 fluorouracil given as intravenous bolus injection in patients with advanced colorectal cancer. They reported comparable response and survival in both groups of patients but a significantly higher incidence of severe diarrhea with the high folinate dose.

In conclusion, our pharmacokinetic/pharmacodynamic results indicate a preference for the lower folinate dose when combined with fluorouracil. Higher exposure to (6S)-folinate increased the incidence of treatment-induced mucositis in patients with advanced colorectal cancer. Minimization of toxicity is of great importance since an acceptable quality of life is one of the major goals of palliative treatment of colorectal cancer. Fluorouracil pharmacokinetics seems to be of minor relevance for toxicity in fluorouracil/folinate regimens. Routine pharmacokinetic monitoring and individualized dosing of fluorouracil might hence be less useful when folinate is coadministered as biomodulator. The significance of nonlinear metabolism of (6S)-folinate for fluorouracil biomodulation remains to be elucidated.

Acknowledgment

The authors thank Dr. Wolfgang Mehnert for his excellent support concerning the statistical analysis.

References

1 Moran RG, Scanlon KL. Schedule-dependent enhancement of the cytotoxicity of fluoropyrimidines to human carcinoma cells in the presence of folinic acid. Cancer Res 1991;51: 4618-23.

2 Kreuser ED, Küchler T, Zwingers T, Matthias M, Ridwelski K, Ehrich PM et al. A multicenter prospective randomized trial with assessment of quality of life in patients with metastatic colorectal carcinoma comparing interferon α-2b and folinic acid as modulators of 5-fluorouracil. Oncology 1994;17:78-84.

3 Cohen AM, Minsky BD, Schilsky RL. Colon cancer. In: De Vita VT, Hellman S, Rosenberg SA (eds) Cancer. Principles & Practice of Oncology. J.B. Lippincott Co., Philadelphia, 1993:929-77.

4 Leichman CG, Leichman L, Spears CP, Rosen PJ, Jeffers S, Groshen S. Prolonged continuous infusion of fluorouracil with weekly bolus leucovorin: A phase II study in patients with disseminated colorectal cancer. J Natl Cancer Inst 1993; 85:41-4.

5 Ardalan B, Chua L, Tian E, Reddy R, Sridhar K, Benedetto P, Richman S, Legaspi A, Waldman S, Morrell L, Feun L, Savaraj N, Livingstone A. A phase II study of weekly 24-hour infusion with high-dose fluorouracil with leucovorin in colorectal carcinoma. J Clin Oncol 1991;9:625-30.

6 Petrelli NJ, Rustum YM, Bruckner H, Stablein D. The Roswell Park Memorial Institute and Gastrointestinal Tumor Study Group phase III experience with the modulation of 5-fluorouracil by leucovorin in metastatic colorectal adenocarcinoma. Adv Exp Med Biol 1988;244:143-55.

7 Petrelli, N, Douglass Jr. HO, Herrera L, Russell D, Stablein DM, Bruckner HW et al. The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. J Clin Oncol 1989;7:1419-26.

8 Jäger E, Heike M, Bernhard H, Klein O, Bernhard G, Lautz D, Michaelis J, Meyer zum Büschenfelde KH, Knuth A. Weekly high-dose leucovorin versus low-dose leucovorin combined with fluorouracil in advanced colorectal cancer: Results of a randomized multicenter trial. J Clin Oncol 1996;14:2274-9.

9 O´Connell MJ. A phase III trial of 5-fluorouracil and leucovorin in the treatment of advanced colorectal cancer. A Mayo Clinic/North Central Cancer Treatment Group study. Cancer 1989;63 (Suppl 6):1026-30.

10 Schalhorn A, Kühl M. Clinical pharmacokinetics of fluorouracil and folinic acid. Semin Oncol 1992;19 (Suppl. 3):82-92.

11 Sips JHM, Van Tellingen O, Nooijen WJ, Rodenhuis S, Ten Bokkel Huinink WW, Beijnen JH. The pharmacokinetics of reduced folates after intraperitoneal and intravenous administration of racemic [6S,R]-folinic acid. Cancer Chemother Pharmacol 1994;35:144-8.

12 Straw JA, Szapary D, Wynn WT. Pharmacokinetics of the diastereomers of leucovorin after intravenous and oral administration to normal subjects. Cancer Res 1984;44:3114-9.

13 Mader RM, Steger GG, Rizovski B, Sieder AE, Locker G, Gnant MFX, Jakesz R, Rainer H. Pharmacokinetics of rac-leucovorin vs [S]-leucovorin in patients with advanced gastrointestinal cancer. Br J Clin Pharmacol 1994;37:243-8.

14 Zittoun J. Pharmacokinetics and in vitro studies of l-leucovorin. Comparison with the d and d,l-leucovorin. Ann Oncol 1993;4 (Suppl 2):S1-S5

15 Streit M, Jaehde U, Stremetzne S, Ridwelski K, Kerz H, Strohbach F, Hohenberger P, Zwiebel FM, Hebart H, Böthig R, Kairies M, Zillig D, Schuchmann S, Warnecke S, Thiel E, Kreuser ED. Five-day continuous infusion of 5-fluorouracil and pulsed folinic acid in patients with metastatic colorectal carcinoma: an effective second-line regimen. Ann Oncol 1997;8:1163-5

16 Christophidis N, Mihaly G, Vajda F, Louis W. Comparison of liquid- and gas-liquid chromatographic assays of 5-fluorouracil in plasma. Clin Chem 1979;25:83-86.

17 Quebbeman EJ, Hoffman NE, Hamid AAR, Ausman RK. An HPLC method for measuring 5-fluorouracil in plasma. J Liq Chromatogr 1984;7:1489-94.

18 Wainer IW, Stiffin RM. Direct resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate using a bovine serum albumin high-performance liquid chromatographic chiral stationary phase coupled to an achiral phenyl column. J Chromatogr Biomed Appl 1988;424:158-62.

19 Comandone A, Bretti S, La Grotta G, Manzoni S, Bonardi G, Berardo R, Bumma C. Palmar-plantar erythrodystesthasia syndrome associated with 5-fluorouracil treatment. Anticancer Res 1993;13:1781-4.

20 Harris BE, Song R, Soong S, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadiane variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res 1990;50:197-201.

21 Metzger G, Massari C, Etienne MC, Comisso M, Brienza S, Toitou Y, Milano G, Bastian G, Misset JL, Levi F. Spontaneous or imposed circadian changes in plasma concentrations of 5-fluorouracil coadministered with folinic acid and oxaliplatin: Relationship with mucosal toxicity in patients with cancer. Clin Pharmacol Ther 1994;56:190-201.

22 Bertrand R, Jolivet J. Lack of interference by the unnatural isomer of 5-formyltetrahydrofolate with the effects of the natural isomer in leucovorin preparations. J Natl Cancer Inst 1989;81:1175-78.

23 Newman EM, Akman SA, Harrison JS, Leong LA, Margolin KA, Morgan RJ, Raschko JW, Somlo G, Ahn CW, Doroshow JH. Pharmacokinetics and toxicity of continuous infusion (6S)-folinic acid and bolus 5-fluorouracil in patients with advanced cancer. Cancer Res 1992;52:2408-12.

24 Schilsky RL, Ratain MJ. Clinical pharmacokinetics of high-dose leucovorin calcium after intravenous and oral administration. J Natl Cancer Inst 1990;82:1411-5.

25 Seifert P, Baker LH, Reed ML, Vaitkevicius VK. Comparison of continuously infused 5-fluorouracil with bolus injection in treatment of patients with colorectal adenocarcinoma. Cancer 1975;36:123-8.

26 Poorter RL, Bakker PJM, Veenhof CHN. Continuous infusion of chemotherapy: focus on 5-fluorouracil and fluorodeoxyuridine. Pharm World Sci 1998;20(2):45-59.

27 Thyss A, Milano G, Renée N, Vallicioni J, Schneider M, Demard F. Clinical pharmacokinetic study of 5-FU in continuous 5-day infusion for head and neck cancer. Clin Pharmacol Ther 1986;16:64-66.