Clin Pharmacokinet
DOI 10.1007/s40262-014-0216-4

ORIGINAL RESEARCH ARTICLE

Kinetically Guided Neoadjuvant Chemoradiotherapy Based on 5-Fluorouracil in Patients with Locally Advanced Rectal Cancer

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Abstract

Background and Purpose This study estimated patients' early response following neoadjuvant chemoradiotherapy (CHRT) of locally advanced rectal cancer based on 5-fluorouracil (5-FU). The target was to achieve pathological complete response (pCR; residual disease-free stage) and toxicities of grade ≤ 2, using individual dosing predicted according to the steady-state plasma concentration (C_{ss}) and pharmacokinetic parameters of 5-FU: the area under the time–concentration curve at steady state (AUC) and clearance (CL).

Patients and Methods This open-label prospective study enrolled 33 adult patients treated with 5-FU administered as a continuous intravenous infusion over 4–5 weeks, as follows: in Group 1a ($N = 6$), the patients received a standard dose of 300 mg/m^2/24 h. In Group 1b ($N = 7$), the patients were treated with an escalated dose of 400–1,000 mg/m^2/24 h. In Group 2 ($N = 20$), the patients were given dosing kinetically guided in order to reach the target range of 5-FU C_{ss} 50–100 μg/L. Tolerability was tested according to Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Radiotherapy was delivered with 10–15 MV photon beams at 1.8 Gy/fraction up to 50.4 Gy in 28 daily fractions for 5 days a week. Surgery followed 4–6 weeks after the completion of CHRT and clinical restaging. The pCR and residual tumour stage were evaluated using preoperative tumour downstaging in magnetic resonance, postoperative histopathological staging and tumour regression rate (residual disease).

Results and Conclusion The cumulative AUC of 5-FU (total exposure to the drug) correlated with cumulative 5-FU dose ($r = 0.61$; $p < 0.001$) and residual disease ($r_s = -0.53$; $p < 0.005$). A higher target pCR rate was reached in patients individually treated (Group 2) who finished the whole 5-week CHRT. The individual daily dose needed to reach the target C_{ss} should be >350 mg/m^2 (up to 600 mg/m^2) provided that 5-FU metabolic ratio is within the range of 2.5–6 and the cumulative AUC5wks is within 50–100 mg·h/L.

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Published online: 11 December 2014

Key Points

Pharmacokinetic/pharmacodynamic target attainment is considered the main principle of neoadjuvant chemoradiotherapy for locally advanced rectal carcinoma based on 5-fluorouracil.

The principal value of 5-fluorouracil pharmacodynamics is to achieve pathological complete response (a residual disease-free stage evaluated according to the TNM grading) and toxicities of grade ≤ 2.

Continuous 7-day intravenous infusion over 5 weeks has been recommended for ambulatory practice.

An individual daily dosing rate within the range of 350–600 mg/m^2 has been predicted in order to maintain a steady-state plasma concentration of 50–100 μg/L and a cumulative area under the time–concentration curve over 5 weeks of therapy of 50–100 mg·h/L, provided the 5-fluoro-5,6-dihydrouracil/5-fluorouracil (inactive metabolite/parent drug) metabolic ratio is within the range of 2.5–6.

1 Introduction

Colorectal cancer is the third most common cancer diagnosis in both sexes. The 5-year survival rate is about 64 %, with a 90 % 5-year survival rate in localized disease (stage I–III) and 10 % 5-year survival rate for patients with tumour metastases spread at time of diagnosis (stage IV) [1, 2]. The cancer stage dictates clinical treatment algorithms and survival, with locally advanced disease warranting multimodality therapy [3]. For stage II–III rectal cancer, preoperative (neoadjuvant) chemoradiotherapy (CHRT) has been widely adopted as the standard of care.

Clinical studies have demonstrated that for stage T3–4, N0, M0 or node-positive adenocarcinoma of the middle and distal rectum, neoadjuvant CHRT based on 5-fluorouracil (5-FU) improves local control and reduces toxicity profiles compared with postoperative (adjuvant) CHRT [4–7]. The incidence of local relapse is lower owing to significant tumour downstaging, downsizing and important changes in histological characteristics [3, 8, 9]. Nodal positivity is less frequent [10] and the rate of sphincter preservation clearly improved [11, 12]. Recently, many studies focused on adjuvant therapy with 5-FU have shown the clear relationship between 5-FU plasma concentrations, toxicity and efficacy in patients treated for advanced colorectal cancer [13–18]. Previous retrospective data have established a link between systemic exposure to 5-FU and treatment-related toxicity rather than dose, related to therapeutic outcome in patients treated for head and neck cancer [19]. This relationship might be useful for an individual 5-FU dose adjustment if neoadjuvant CHRT of locally advanced rectal cancer is recommended.

The main inactive metabolite of 5-FU in plasma is dihydroderivative, i.e. 5-fluoro-5,6-dihydrouracil (5-FUH2) [13–18]. The amount of inactive metabolite is in excess compared to the parent drug (usually about 3–5 times higher concentration of 5-FUH2 compared to 5-FU). The ratio of 5-FUH2/5-FU concentrations is called 'metabolic ratio' [18]. 5-FU is then undergoing intracellular conversion via a number of intermediates to 5-fluoro-2-deoxyuridine 5′-monophosphate (FdUMP), an inhibitor of thymidylate synthase, 5-fluoro-2-deoxyuridine 5′-triphosphate (5dUTP), which can be falsely incorporated into DNA, and 5-fluorouridine 5′-triphosphate (FUTP), which can inhibit RNA synthesis. As an antimetabolite, 5-FU is both S-phase and cycle specific. It follows that prolonged exposure to the drug is likely to be more effective if given when the vast majority of cancer cells will be out of cycle [17]. 5-FU has an extremely low value of half-life of elimination [$t_{½}$] (i.e. 5–10 min) [16–18, 20–23]. That is why 5-FU steady-state plasma concentration (C_{ss}) is reached after 30–60 min (usually <2 h).

The main objective of this study, therefore, was to estimate pharmacokinetic/pharmacodynamic relationship as follows:

The patients' early response to the treatment (tumour downstaging, downsizing and important changes in histological characteristics) and its correlation to individual 5-FU plasma concentrations and pharmacokinetic parameters of 5-FU: is kinetically guided neoadjuvant therapy with 5-FU, i.e. individual dosing rate, predicted in respect to individual plasma concentration and pharmacokinetic parameters?

Secondary objectives were focused on evaluation of tolerability of such a regimen when compared to the standard concomitant chemotherapy and irradiation.

For this reason, the fixed-dose therapy (both standard dosing and dose escalation) and individual 5-FU dosing adjustment was used. This approach aimed to find the relationship between the 5-FU dosing rate on one hand and both the target C_{ss} (or area under the time–concentration curve at steady state [AUC]) and signs of capacity-limited elimination on the other hand. *The first aim* was to reach the target 5-FU C_{ss} of 50–100 μg/L over 7-day therapy. This range was derived from that considered the threshold value for adjuvant therapy limited by higher risk for leukopenia, diarrhoea and mucositis [20, 21, 24]. *The second aim* was to avoid toxicity (grade ≥ 2).

2 Patients and Methods

This open-label prospective study enrolled patients who received neoadjuvant CHRT at the Department of Oncology and Radiotherapy, University Hospital, Hradec Králové, Czech Republic between January 2009 and December 2011. Surgery was performed at the Department of Surgery, University Hospital. The study protocol was approved by the Ethics Committee of University Hospital and all patients provided written informed consent. The inclusion criteria were confirmed as locally advanced, histologically confirmed, previously untreated rectal adenocarcinoma of the middle and distal rectum T3–4, N0–2, M0 (Table 1). Additional enrolment criteria included haematocrit >29 %; plasma haemoglobin >100 g/L; absolute white blood cell count >3,000 cells/μL and neutrophil count >1,500 cells/μL; platelet count >100,000 cells/μL, serum creatinine <15 μmol/100 mL; and aspartate aminotransferase activity <2 times the institutional upper limit of normal values. Only the patients who completed the full course of neoadjuvant therapy were included in the final evaluation. Patients were excluded for uncontrolled arterial hypertension, therapeutic anticoagulation use, pregnancy or nursing, the need for urgent surgery and multiple missing data points in the database.

Preoperative radiotherapy was delivered with 10–15 MV photon beams at BOX technique, 4 fields. The prescribed dose was specified according to the guidelines of the International Commission on Radiation Units and Measurements Reports. Proximally, the target volume was extended to the sacral promontory. Posteriorly, the target volume reached the ischial tuberosity. Laterally, the target volume was extended to the pelvic sidewalls. Its posterior margin included all presacral soft tissue, i.e. the anterior border of the sacrum with at least 1.5 cm limit. Anteriorly, the lateral target covered the posterior border of the vagina or that of prostate, and the anterior extent of the both primary rectal tumour and anterior edge of the sacral promontory.

2.1 Pretreatment Stage

All patients underwent a complete medical history, physical and digital rectal examinations, a transrectal rectoscopy with biopsy, a full colonoscopy and a computed tomography (CT) scan of chest, abdomen and pelvis. The distance between the tumour distal border and the anal verge was determined by rectoscopy. The T-stage was primarily defined by the transrectal ultrasound and completed by magnetic resonance. Every visible lymph node in the transrectal ultrasound and/or in the CT scan was classified as positive [25, 26].

2.2 5-FU Dosing

2.2.1 Fixed-Dose Therapy

Standard Dosing—Group 1a: 5-FU 300 mg/m^2/24 h for 7 consecutive days over weeks 1–5 was administered (i.e. 5 weeks of unmodified chemotherapy).

Dose Escalation—Group 1b: Chemotherapy was initiated with 5-FU 400 mg/m^2/24 h for 7 consecutive days over week 1 which was gradually increased by 200 mg/m^2/24 h weekly, i.e. in week 2, 500 mg/m^2/24 h; in week 3, 700 mg/m^2/24 h; and in week 4, 1,000 mg/m^2/24 h provided that no evidence of significant (grade >2) toxicity became apparent. This approach aimed to find the relationship between the 5-FU dosing rate on one hand and

Table 1 Evaluation of tumour regression—clinically and pathologically (in accordance with references [25] and [26])

Evaluation scale (0–12)	cTcNM—clinical evaluation before chemoradiotherapy	ycTycNM—clinical evaluation 4 weeks after chemoradiotherapy	ypTypNM—pathological evaluation after tumour resection
0	–	cT0cN0M0	ypT0ypN0M0
1	–	cT1cN0M0	ypT1ypN0M0
2	–	cT1cN1M0	ypT1ypN1M0
3	–	cT1cN2M0	ypT1ypN2M0
4	T2N0M0	cT2cN0M0	ypT2ypN0M0
5	T2N1M0	cT2cN1M0	ypT2ypN1M0
6	T2N2M0	cT2cN2M0	ypT2ypN2M0
7	T3N0M0	cT3cN0M0	ypT3ypN0M0
8	T3N1M0	cT3cN1M0	ypT3ypN1M0
9	T3N2M0	cT3cN2M0	ypT3ypN2M0
10	T4N0M0	cT4cN0M0	ypT4ypN0M0
11	T4N1M0	cT4cN1M0	ypT4ypN1M0
12	T4N2M0	cT4cN2M0	ypT4ypN2M0

both the target C_{ss} (or AUC) and signs of capacity-limited elimination on the other hand.

2.2.2 Individual 5-FU Dosing Adjustment

Group 2: The patients were given a standard 5-FU dose of 400 mg/m^2/24 h for 7 consecutive days over week 1. Starting in week 2, the dosing was modified according to the individual 5-FU pharmacokinetics and toxicity. If plasma 5-FU C_{ss} exceeded 200 μg/L the infusion was stopped for 1–2 weeks and started with 50 % reduction of dose according to 5-FU toxicity. If 5-FU C_{ss} was about 80–100 μg/L, the dose delivery was unchanged. If the 5-FU C_{ss} was <50 μg/L, the dose was escalated about 50 %, in all cases regarding 5-FU toxicity.

5-FU doses were administered as a continuous intravenous infusion for 7 consecutive days over 4–5 weeks [27, 28] via an implanted port catheter to the jugular or subclavian vein in order to provide the delivery of chemotherapy at home (intensified ambulatory practice). After 4–6 weeks, when the completion of CHRT and clinical restaging had been finished, surgical resection with a curative aim including the total mesorectal excision or rectum amputation was performed [11].

2.3 Pathological Response Evaluation and Residual Tumour Stage (Residual Disease)

The response to CHRT, i.e. the presence (progression, regression or stabilization of the disease) or absence of any residual disease was evaluated 4–6 weeks after CHRT by means of tumour downstaging (clinically), histopathological staging and tumour regression rate. Clinical staging (cTcNM) and pathological staging evaluation (ypTypNM) were recorded according to rules given in the Union for International Cancer Control (UICC) Tumour–Node–Metastasis (TNM) system. Tumour downstaging was defined by the comparison of the pretreatment cT and cN clinical stage to the post-treatment clinical stage, i.e. ycT and ycN; the stages were determined by magnetic resonance of the rectum in both periods (Table 1). After surgery, histopathological staging was recorded using UICC criteria as pTpNM classification [29]. The tumour regression rate was based on initial tumour spread (cTcNM results, preoperatively) compared to ypTypNM (examined by the pathologist postoperatively). The target response was to reach the absence of residual disease (i.e. ypT0ypN0)—a pathological complete response (pCR)—known to be the predictive factor of better overall survival examined during the follow-up period (post-treatment), i.e. 2–5 years after therapy termination.

2.4 Blood Sampling and 5-FU Determination

Blood sampling twice a week was dictated by the intensified ambulatory practice (the patients were not hospitalized but they arrived every working day for irradiation from Monday to Friday). Every week, therefore, the blood samples were drawn on days 2 and 5 between 8:00 and 10:00 a.m. (i.e. about 24 and 96 h after the start of a 7-day continuous infusion on day 1).

Blood samples were collected into tubes with EDTA and immediately centrifuged (10 min, 4 °C, 1,000×*g*). Plasma supernatants were stored at 4 °C and transferred to the laboratory, where they were kept at −80 °C for a maximum of 14 days until high-performance liquid chromatography (HPLC) analysis for 5-FU and its metabolite was performed.

2.4.1 Sample Preparation

Samples of frozen plasma were thawed at room temperature, protected from light and subjected to a solid-phase extraction (SPE) on the Atoll XC cartridges (100 mg, 3 mL; Interchim, Montluçon, France). The procedure was as follows: conditioning with 2 mL of acetonitrile, 2 mL of

Table 2 Lower limit of quantification (LLOQ) precision and accuracy of the method according to quality control (QC)

Variable	5-FUH2				5-FU			
	LLOQ 50 ng/mL	QC1 120 ng/mL	QC2 500 ng/mL	QC3 1,000 ng/mL	LLOQ 20 ng/mL	QC1 60 ng/mL	QC2 300 ng/mL	QC3 600 ng/mL
Precision (CV%)								
Intra-day ($n = 5$)	10.01	5.21	1.41	2.00	6.75	1.68	1.23	2.18
Inter-day ($n = 11$)	–	8.40	4.68	2.31	–	4.23	2.02	1.52
Accuracy (RE%)								
Intra-day ($n = 5$)	16.8	13.31	3.51	8.88	−2.52	−0.44	−0.85	1.09
Inter-day ($n = 11$)	–	8.53	4.26	10.99	–	−3.37	−1.7	0.77

5-FU 5-fluorouracil, *5-FUH2* 5-fluoro-5,6-dihydrouracil (the inactive metabolite), *CV* coefficient of variation, *RE* relative error

methanol, 1 mL of MilliQ water and 1 mL of phosphate buffer (KH_2PO_4, $c = 10$ mmol/L, pH = 2.0). Plasma aliquot 1.5 mL was mixed with 60 µL of an internal standard (5-chlorouracil, $c = 10$ µg/mL), transferred to the conditioned SPE cartridge and allowed to pass the cartridge at a speed of ~25 µL/s. Washing was carried out with 1 mL of ammonium formate buffer (10 mmol/L, pH = 5.0). Elution was carried out with 0.5 mL of a solution composed of acetonitrile:methanol (20:80, v/v). Eluates were evaporated in thermoblock under stream of nitrogen (45 °C, 15 min), then reconstituted in 200 µL of 10 mmol/L hydrochloric acid and subjected to centrifugation (14,000×*g*, 5 min). Aliquot of 100 µL was injected on the HPLC column.

2.4.2 Chromatographic Conditions

All analyses were performed on a 1200 series Agilent liquid chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) composed of a degasser, quaternary pump, light-tight autosampler unit set at 15 °C, thermostated column compartment held at 25 °C and a UV/VIS detector with following wavelength programme: 0–9 min at 210 nm, 9–14 min at 260 nm and 14–19 min at 210 nm. Separation was carried out on a Luna PFP [2] reverse phase column (150 × 4.6 mm I.D., 5 µm particle size), protected with a Luna PFP 4 × 3 mm guard column (Phenomenex; Torrance, CA, USA) at a temperature of 25 °C. For the separation, a gradient elution was used with mobile phases composed of MilliQ water at pH = 2.5, acidified with sulphuric acid (solvent A) and solvent B composed of methanol mixed with solvent A (1:1, v/v). Prior to use, the mobile phase was filtered through a 0.22 µm Durapore filter (Millipore; Milford, MA, USA). The gradient elution programme was as follows: 0–5.5 min with 6 % of solvent B, 5.5–12 min with a linear gradient 6–36 % of solvent B, 12–14 min with solvent B at 100 % and 14–19 min equilibration at 6 % of solvent B. During the equilibration, the next sample was prepared for injection, thus the total analysis runtime was 19 min. Retention times of compounds were as follows: 5-FUH2 $t_r = 5.58$ min, 5-FU $t_r = 6.34$ min, 5-chlorouracil (internal standard) $t_r = 10.72$ min. The method was found to be linear in the range of 50–2,000 ng/mL for 5-FUH2 and 20–2,000 ng/mL for 5-FU. Limits on the low end of the ranges are also lower limits of quantification (LLOQs). The precision and accuracy of the method met the US FDA criteria according to the Guidance for Industry, Bioanalytical Method Validation (Table 2).

2.5 Pharmacokinetics

The total (plasma) clearance was calculated as the 5-FU dosing rate divided by the steady-state plasma concentration: CL_{tot} = dosing rate/C_{ss}. The AUC of 5-FU was calculated as C_{ss} multiplied by infusion duration (T_{CI}), i.e. 168 h (AUCwk) for each therapy week as follows: AUCwk = $C_{ss} \times T_{CI}$. Cumulative AUC was the sum of the AUCs obtained in four or five therapy weeks (depending on duration of therapy—AUC4wks, AUC5wks). The 5-FUH2/5-FU metabolic ratio was calculated.

2.6 Treatment Tolerability and Follow-Up

Treatment-induced toxicity was assessed by the Common Terminology Criteria for Adverse Events v3.0 (CTCAE). The following parameters were recorded and evaluated:

(a) Perianal post-irradiation dermatitis in 4-point scale (1—painless erythema, 2—painful erythema, 3—erythema with desquamation, 4—life-threatening, disabling);
(b) Diarrhoea in 4-point scale: 1. <4 stools per day over baseline; 2. 4–6 stools per day over baseline; 3. ≥7 stools per day over baseline or incontinence; 4. life threatening consequences—i.e. haemodynamic collapse.

Finally, the score of toxicity grade in week 5/week 1 was evaluated. In case of severe toxicities, 5-FU dosing was reduced or suspended.

2.7 Statistical Evaluation

The influence of the week of therapy on pharmacokinetic/pharmacodynamic characteristics was assessed using analysis of variance (ANOVA) for repeated measures. Parametric ANOVA followed with the Tukey's test or nonparametric Kruskal–Wallis ANOVA and Dunn's post test were used as appropriate. Spearman's correlation coefficient was used to evaluate the relationships between the variables under study. Calculations were performed with the help of GraphPad Prism 5.00 for Windows (GraphPad Software; San Diego, CA, USA).

3 Results

3.1 Patients' Characteristics

The study period extended from January 2009 to December 2011. A total of 39 patients affected by rectal adenocarcinoma were enrolled in the trial (Table 3). Thirty-three of the 39 patients met all requirements of the study protocol and completed the neoadjuvant therapy as follows: 30/33 (91 %) completed the 4-week CHRT, and 21/33 (64 %) completed the 5-week CHRT.

Table 3 Characteristics of patients—initial staging of disease according to Tumour–Node–Metastasis (TNM) system

Characteristic	All groups	Group 1a	Group 1b	Group 2
Number of patients	33	6	7	20
Age, mean (SD) (years)	66.0 (6.3)	68.2 (6.8)	66.2 (6.3)	64.6 (10.4)
Male/female, *n* (%)	27/6 (82/18)	3/3 (50/50)	6/1 (86/14)	18/2 (90/10)
TNM, *n* (%)				
T2N1M0	1 (3)	0 (0)	0 (0)	1 (5)
T3N0M0	6 (18)	1 (17)	2 (28)	3 (15)
T3N1M0	6 (18)	2 (34)	1 (14)	3 (15)
T3N2M0	11 (33)	0 (0)	3 (42)	8 (40)
T4N2M0	9 (27)	3 (51)	1 (14)	5 (25)

SD standard deviation

Three groups of patients were stratified as follows:

Group 1 ($n = 13$), males/females 9/4, age 67.8 ± 6.5 years: Six of the 13 patients were treated with 5-FU standard dose of 300 mg/m^2/24 h for 7 consecutive days over weeks 1–5 (Group 1a); 7/13 patients were treated with escalated dosing 400–1,000 mg/m^2/24 h for 7 consecutive days as follows: 400 mg/m^2/24 h in week 1, 500 mg/m^2/24 h in week 2,700 mg/m^2/24 h in week 3 and 1,000 mg/m^2/24 h in week 4 (Group 1b).

Group 2 ($n = 20$), males/females 18/2, age 64.6 ± 10.4 years: Chemotherapy was started with 5-FU 400 mg/m^2/24 h for 7 consecutive days in week 1. The daily dose for weeks 2–5 was individualized in order to reach the target 5-FU C_{ss} (50–100 μg/L) and toxicity grade (0–2). 5-FU dosing did not exceed 600 mg/m^2/24 h to avoid toxicity.

Radiotherapy was given for 5 consecutive days over weeks 1–4 or weeks 1–5.

3.2 5-FU Pharmacokinetics/Pharmacodynamics

Pharmacokinetic parameters of 5-FU estimated in patients in Groups 1a, 1b and 2 are given in Tables 4, 5 and 6.

Pharmacokinetic parameters of 5-FU estimated for all 33 patients are as follows: mean (standard deviation) AUCwk values of 5-FU in patients treated over weeks 1–4, respectively: 11.7 (5.8), 13.8 (5.5), 16.2 (10.7) and 15.1 (11.9) mg·h/L. Similar results were achieved for the 5-week therapy, i.e. 12.9 (6.1), 13.1 (5.6), 14.9 (9.6), 13.1 (7.9) and 13.8 (7.8) mg·h/L in those treated for weeks 1–5, respectively.

In weeks 1–5, the daily dose (mg/m^2) of 5-FU was correlated with 5-FU plasma concentration and AUCwk as follows: week 1: $r = 0.36$, $p = 0.04$; week 2: $r = 0.52$, $p < 0.005$; week 3: $r = 0.45$, $p < 0.011$; week 4: $r = 0.79$, $p < 0.001$; week 5: $r = 0.40$, $p < 0.002$.

The 5-FU AUCwk values were not dependent on a week of treatment and reached 48–62 % of the equivalent threshold AUC value associated with the incidence of toxicity.

There is a significant relationship between the cumulative dose and the cumulative AUC (AUC4wks and AUC5wks; $r_s = 0.61$, $p < 0.001$) [Fig. 1]. Seventeen patients (53 %) reached a cumulative AUC within the range of 50–100 mg·h/L, 12 patients (38 %) reached <50 mg·h/L and three patients (9 %) exceeded the value of 100 mg·h/L.

The mean value of 5-FU clearance reached 216–248 L/h/m^2 over weeks 1 through 5. There was a wide variability in this value. In patients treated with CHRT for 4 weeks,

Table 4 Pharmacokinetic parameters of 5-fluorouracil (5-FU) in patients treated with 5-FU 300 mg/m^2/24 h for 7 consecutive days in 5 weeks (Group 1a; $N = 6$)

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Cumulative parameter
C_{ss} (ng/mL)	55.7 (37.7)†	82.6 (29.9)	74.4 (18)	77.7 (20)	82.3 (8.5)†	–
AUCwk (mg·h/L)	10.0 (2.3)‡	10.0 (2.4)	10.7 (2.0)	11.2 (2.4)	11.5 (2.3)‡	64.2 (17.2)
CL (L/h)	189 (44)	191 (45)	175 (34)	191 (46)	163 (36)	–
Metabolic ratio (5-FUH2/5-FU)	5.2 (3.2)†	4.7 (1.7)	4.1 (1.8)	3.6 (1.9)	2.3 (1.2)†	–

Data are given as mean (standard deviation); † $P < 0.01$, week 5 versus week 1; ‡ $P < 0.05$, week 5 versus week 1

C_{ss} steady-state plasma concentration, *AUCwk* area under the time–concentration curve of 5-FU estimated for 7-day infusion, *CL* 5-FU clearance, *5-FUH2* 5-fluoro-5,6-dihydrouracil (the inactive metabolite)

Table 5 Pharmakokinetic parameters of 5-fluorouracil (5-FU) in patients treated with 5-FU dose escalation 300–1,000 mg/m^2/24 h for 7 consecutive days in 4 weeks (Group 1b; $N = 7$)

Parameter	Week 1 300 mg/m^2/24 h	Week 2 500 mg/m^2/24 h	Week 3 700 mg/m^2/24 h	Week 4 1,000 mg/m^2/24 h	Cumulative parameter
C_{ss} (ng/mL)	59.2 (14.6)†	102.4 (24.1)	201.4 (59.3)	284.5 (112.9)†	–
AUCwk (mg·h/L)	8.5 (1.7)†	14.8 (2.9)	29.0 (6.7)	40.9 (13.5)†	62.0 (33.8)
CL (L/h)	218 (54)	210 (50)	190 (132)	159.0 (63)	–
Metabolic ratio (5-FUH2/5-FU)	5.5 (2.8)†	3.5 (2.0)	4.3 (2.2)	3.8 (2.4)†	–

Data are given as mean (standard deviation); † $P < 0.01$, week 4 versus week 1

AUCwk area under the time–concentration curve of 5-FU estimated for 7-day infusion, *CL* 5-FU clearance, C_{ss} steady-state plasma concentration, *5-FUH2* 5-fluoro-5,6-dihydrouracil (the inactive metabolite)

Table 6 Pharmacokinetic parameters of 5-fluorouracil (5-FU) in patients individually treated (initial daily dose of 5-FU 400 mg/m^2/24 h) (Group 2; $N = 20$)

Parameter	Week 1	Week 2	Week 3	Week 4	Week 5	Cumulative parameter
C_{ss} (ng/mL)	84.0 (49.5)	72.9 (29.1)	91.8 (58.6)	78.1 (44.2)	94.9 (51.9)	–
AUCwk (mg·h/L)	14.1 (8.3)	12.2 (4.9)	15.4 (9.8)	13.1 (7.4)	15.9 (8.7)	69.4 (26.7)
CL (L/h)	240 (142)	246 (138)	213 (166)	241 (139)	198 (103)	–
Metabolic ratio (5-FUH2/5-FU)	3.7 (1.9)	3.5 (2.1)	3.7 (1.8)	4.1 (2.1)	3.9 (2.5)	–

Data are give as mean (standard deviation)

AUCwk area under the time–concentration curve of 5-FU estimated for 7-day infusion, *CL* 5-FU clearance, C_{ss} steady-state plasma concentration, *5-FUH2* 5-fluoro-5,6-dihydrouracil (the inactive metabolite)

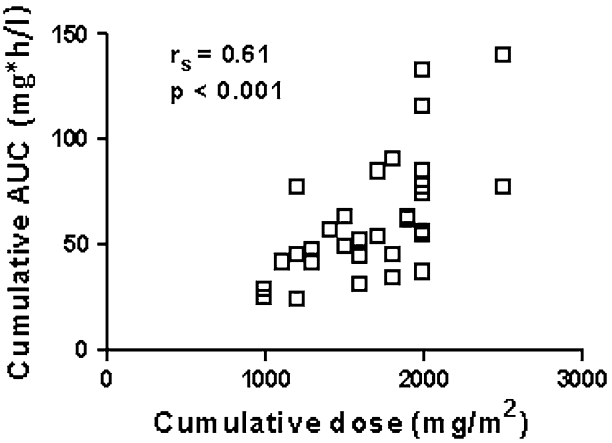

Fig. 1 Correlation of 5-FU cumulative dose (mg/m^2) given by intravenous continuous infusion over 4–5 weeks with the cumulative 5-FU $AUC_{4–5\ weeks}$ value

this parameter was characterized by both intraindividual variability (coefficient of variation [CV] 43 %) and interindividual variability (CV 65 %). Similarly, in those treated for 5 weeks, wide intraindividual and interindividual variability was found (CV 46 and 78 %, respectively). Intraindividual variability reached 65 % of interindividual variability in the former and 58 % interindividual variability in the latter. The 5-FU clearance was not influenced either by the age, sex, daily dose or week of therapy. The decrease in the 5-FU clearance over weeks 1 through 4 and 5 did not reach statistical significance; there was registered only a trend towards a decrease (ANOVA; $r_s = 0.066$) [Tables 4, 6].

The surgical procedures followed in weeks 4 through 6 after CHRT termination included abdominoperineal resection with sphincter preservation in 24 patients (72 %; in 8 patients with transient colostomy) and amputation of rectum in 9 patients (27 %).

After the CHRT termination and surgery, each patient's response was evaluated and expressed in respect to cumulative AUC and stage of residual disease (pCR = no residual disease, i.e. the target value). The results (Figs. 2, 3) demonstrate a significant relationship between the cumulative AUC (exposure to 5-FU over complete CHRT) and pathological tumour regression ($r_s = -0.53$, $p < 0.001$).

Patients' response compared to cumulative AUC was evaluated in 33 individuals. Fourteen patients with 5-FU exposure <50 mg·h/L were at a high risk of therapeutic failure. Sixteen patients reached a cumulative AUC within the target range of 50–100 mg·h/L and 6/16 patients experienced pCR; 5/6 patients who experienced pCR were individually treated (Group 2).

Patients' Response in Relation to Standard/Kinetically Guided Therapy: *In Group 1* (Group 1a, i.e. 5-FU 300 mg/m^2, and Group 1b, i.e. 5-FU dose escalated), 6/13 patients (46 %) finished the 5-week CHRT and only 1 reached the

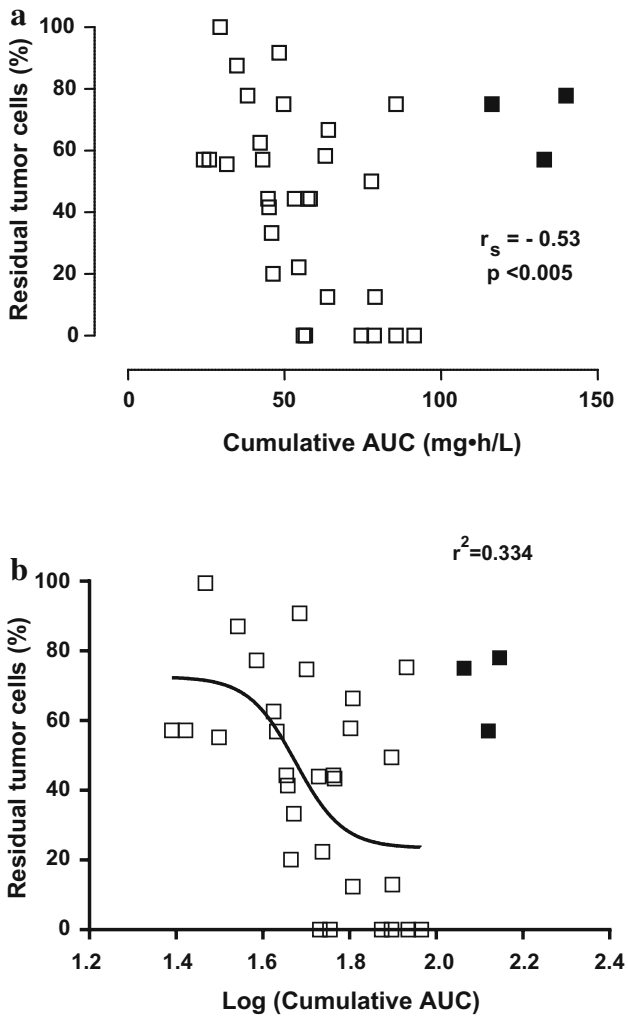

Fig. 2 **a** Linear relationship between the cumulative area under the time–concentration curve at steady state (AUC) [exposure to 5-fluorouracil over chemoradiotherapy] and pathological tumour regression. Six patients with cumulative AUC within 50–100 mg·h/L reached the target pathological complete response. *Three black squares* demonstrate outcome of three patients (one from Group 1b treated with escalated doses, two from Group 2) characterized by a prolonged period (>2 months) between chemoradiotherapy completion and surgical resection owing to serious toxicity after a higher daily dose. **b** S-shaped AUC–response curve as a result from assessing relationship between the cumulative AUC and pathological tumour regression

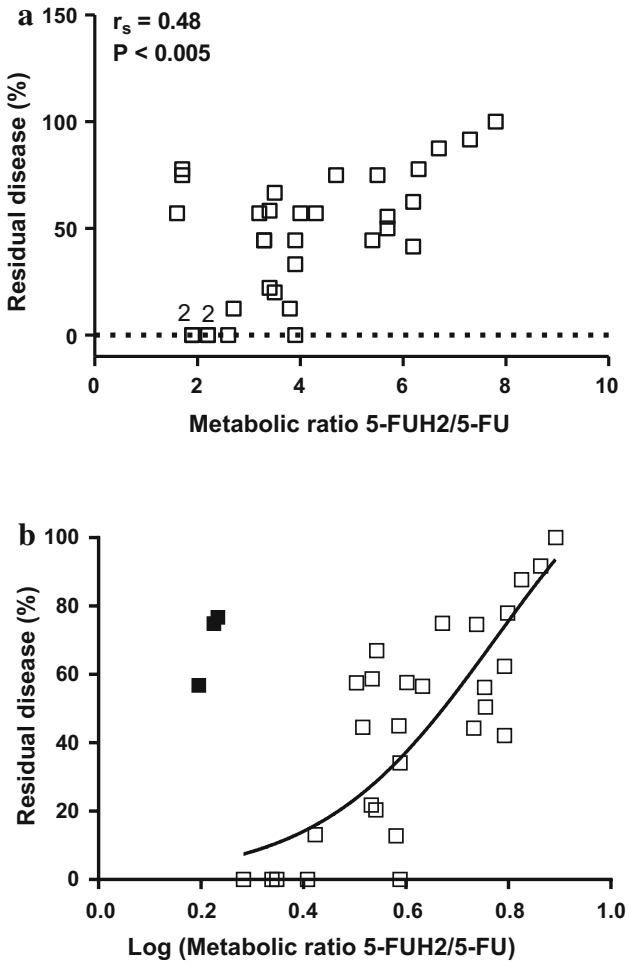

Fig. 3 **a** Linear relationship between 5-fluoro-5,6-dihydrouracil/5-fluorouracil (5-FUH2/5-FU) metabolic ratio and residual disease. *Three black squares* demonstrate outcome of three patients (one from Group 1b treated with escalated doses, two from Group 2) [see Fig. 2] with very low metabolic index, who were suffering from serious toxicity and therapeutic failure. **b** Relationship between 5-FUH2/5-FU metabolic ratio and pathological tumour regression described by S-shaped metabolic ratio–response curve

target pathological response; 6/13 patients underwent a 4-week CHRT and one patient (1/13) underwent a 3-week CHRT.

In Group 2 (kinetically guided therapy), 15/20 patients (75 %) finished the 5-week CHRT; 5/15 (33 %) achieved pCR; 3/20 patients underwent a 4-week CHRT and 2 patients (2/20) underwent a 3-week CHRT. There was a statistically significant difference in efficacy of CHRT (i.e. the rate of decrease in pT pN classification versus T and N evaluation before CHRT) between the subgroup of 15/20 patients finishing the 5-week CHRT and 5/20 patients who did not ($p = 0.04$; Mann–Whitney nonparametric test).

Moreover, *there was a statistically significant difference in efficacy of CHRT* between Groups 1a and 2 after omission of 3 subjects with severe toxicity and postponed surgery ($p = 0.03$; Mann–Whitney nonparametric test). These patients experienced both a high cumulative AUC (>100 mg·h/L) [Figs. 2, 3] and a low metabolic ratio (<1.8) but without reaching the target response. One of three patients (Group 1b) was administered a high 5-FU dose (>600 mg/m^2/24 h), 2/3 patients (Group 2) were given an individualized dose. In spite of this difference, all three patients developed severe toxicity which resulted in postponed surgery: one patient underwent tumour resection 18 weeks later after CHRT because of severe

Table 7 Tolerability of neoadjuvant chemoradiotherapy based on 5-fluorouracil (5-FU) in patients treated with dosing rate 300–1,000 mg/m^2/24 h (Group 1a, 1b; $N = 13$)

Parameter	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 5/Week 1
Haemoglobin (g/L)	135.2 (17.4)	130.2 (17.6)	132.8 (17.8)	124.6 (16.9)	126.5 (14.8)	122.8 (15.3)	−12.4
Post-irradiation reaction (0–4)	0	0.23 (0.32)	0.87 (0.51)	1.56 (0.77)	1.78 (0.83)	1.94 (0.59)	+1.71
Grade >2 (%)		0	8	16	24	32	
Diarrhoea (0–4)	0	0.34 (0.43)	0.94 (0.53)	1.59 (0.58)	1.97 (0.67)	2.37 (0.79)	+2.03
Grade >2 (%)		0	8	16	24	32	

Data are given as mean (standard deviation)

diarrhoea and ulcerative necrotic enteritis. Two of three patients underwent tumour resection 12 weeks later for severe perianal dermatitis which appeared 2 weeks after CHRT termination. Surprisingly and in contrast to others, these patients confessed to taking folinic acid as a supplement at a dose of 2.5–5 mg a day over the study. As they did not meet the rules of the study protocol, their outcomes regarding both CHRT efficacy and toxicity were not included in the study evaluation.

Patients' Response and Metabolic Ratio: The 5-FUH2/5-FU metabolic ratio was negatively correlated with residual disease. In the left top corner, three squares demonstrate very low metabolic ratio (<1.8) found in noncompliant patients mentioned above. The relationship between both residual tumour versus cumulative AUC and metabolic ratio follows a concentration–response sigmoid curve with a linear midportion (Fig. 3). Both the former and the latter are located out of the linear midportion. This fact implies the importance of individual pharmacokinetic/dynamic approach in order to achieve the target response with acceptable toxicity (grade ≤2).

3.3 Treatment Tolerability of Neoadjuvant CHRT Based on 5-FU

Haematological Toxicity: Grade 3/4 neutropenia was seen in one patient after a 5-FU high dose (>600 mg/m^2/24 h) in week 6, complicated by enterocolitis, lasting for 7 days and treated by intravenous metronidazole 500 mg every 6 h for 10 days (Group 1). Grade 1–2 anaemia was diagnosed in 15 patients (15 %); thrombocytopenia was absent.

Nonhaematological Toxicity: The occurrence of grade >2 nonhaematological toxicity was examined (Tables 7, 8). One patient (Group 1b) developed enterocolitis (see above). Nine out of 10 patients suffering from grade >2 diarrhoea were treated with loperamide 6–12 mg a day orally (6 of these patients were from Group 1, and 3 patients were from Group 2).

The toxicity grade of diarrhoea, skin reaction and local (perianal) pain was gradually increasing every week of the CHRT, while transaminase activity and anaemia were not. Toxicity was fully reversible in all patients.

4 Discussion

The target values for kinetically guided treatment have been established in clinical trials focused on *head and neck cancer*, and a simple dose adjustment for 5-FU adjuvant chemotherapy has been developed, enabling easy adoption of the practice in clinical settings. These studies underline the importance of both C_{ss} and systemic exposure to 5-FU (AUC). In order to achieve optimal exposure, a reliable pharmacokinetically guided protocol was declared beneficial [30, 31]. Simple, individual-patient 5-FU pharmacokinetic monitoring with dose adjustment was more effective in ensuring an appropriate treatment course with minimized toxicity and optimized outcome. Dosing rate was considered an important predictor of therapeutic outcome for a variety of different tumour types because patients treated with inadequate doses of chemotherapy are relatively disadvantaged by both therapeutic failure and severe toxicity [14].

Contributing to neoadjuvant CHRT for locally advanced rectal cancer based on 5-FU we aimed to:

1. Assess the variability of 5-FU C_{ss} and pharmacokinetic parameters within the study population and correlate pharmacodynamics/toxicodynamic end points, such as early antitumour response and toxicity, with the 5-FU steady-state pharmacokinetics in individual patients.
2. Adjust the individualized 5-FU dosing according to the patient's plasma concentrations and 5-FU pharmacokinetic parameters.

Mode of 5-FU administration and dosing rate was the first question to answer.

Cytostatic therapy has been based on continuous intravenous infusion of 5-FU. C_{ss} develops within 2 h after the start of intravenous infusion to achieve the continuous 5-FU exposure (AUC)—an important determinant of

Table 8 Tolerability of neoadjuvant chemoradiotherapy based on 5-fluorouracil (5-FU) in patients treated with kinetically guided dosing rate (Group 2; $N = 20$)

Parameter	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 5/Week 1
Haemoglobin (g/L)	144.6 (12.5)	142.6 (18.4)	140.0 (11.0)	138.5 (11.9)	136.8 (14.1)	134.1 (14.2)	−10.45
Post-irradiation perianal reaction (0–4)	0	0.22 (0.18)	0.45 (0.24)	0.68 (0.35)	0.98 (0.49)	1.04 (0.53)	+0.82
Grade >2 (%)		0	0	0	0	0	
Diarrhoea (0–4)	0	0.20 (0.15)	0.35 (0.12)	0.55 (0.39)	0.90 (0.55)	1.20 (0.73)	+1.00
Grade >2 (%)		0	0	5	10	15	

Data are given as mean (standard deviation)

cytotoxicity. Individual steady-state pharmacokinetic parameters are easy to estimate. Such a mode of 5-FU administration is known to induce a response superior to that induced by bolus injection and to shift the usual limiting toxicity from myelosuppression to stomatitis and gastrointestinal toxicity [32, 33]. On the other hand, owing to the very short $t_{½}$, the continuous exposure to 5-FU should be carefully maintained without interruption [34, 35].

Prior to the start of this study, five patients were administered 5-FU 200 mg/m^2/24 h for a 5-day cycle (not shown). The outcome indicated 5-FU C_{ss} to be very low. We decided, therefore, to increase 5-FU dosing rate using 300 mg/m^2/24 h in 7-day cycles over 4–5 weeks. This mode of administration (fixed-dose therapy, Group 1a) was technically simpler and easy to monitor in the setting of ambulatory practice. No serious symptoms of toxicity were noticed.

Capacity-limited elimination which becomes saturable with increasing dosing rate was the second question we had to answer prior to dose adjustment according to individual 5-FU pharmacokinetics. Therefore, a dose escalation schedule (Group 1b) was used to imply increasing the dose from a nontoxic level to an 'acceptable toxic level'. This approach should reveal saturable pharmacokinetics possibly related to higher dosing rate. Indeed, in patients treated with highest 5-FU dosing (500–700 mg/m^2/24 h) a 40 % increase in dose resulted in 100 % increase in C_{ss} and AUC, respectively (Table 5). 5-FU dosing was associated with toxicity (diarrhoea, leukopenia, perianal dermatitis); nevertheless, every sign of toxicity was reversible.

Variability of 5-FU Pharmacokinetic Parameters: In accordance with others [18, 36], end points of Group 1a and 1b confirmed wide intra- and interindividual variability in the 5-FU pharmacokinetic parameters including 5-FU clearance. This finding is important for clinical practice because in chemotherapy, the value of drug total and/or renal clearance is often considered predictive for both efficacy and toxicity. For therapy with 5-FU, total clearance can hardly be considered predictive for 5-FU exposure unless this parameter is individually estimated at the start of every week.

Dose and 5-FU Pharmacokinetics/Pharmacodynamics: For the reason mentioned above, we have chosen, therefore, another predictive criterion for attaining the target response. That is cumulative AUC justified as follows:

- There were no significant differences in this parameter between cycles 5 and 1 or between cycles 4 and 1.
- In accordance with the literature analysing adjuvant therapy with 5-FU [22, 23], we found a significant positive relationship between the response to CHRT and 5-FU pharmacokinetics (5-FU C_{ss} and the cumulative AUC) within the dosing range (a window) characterized by acceptable toxicity.

The 5-FU pharmacokinetic/pharmacodynamic relationship, therefore, has been used for *predicting the dosing schedule* based on the individual pharmacokinetic parameters (Group 2). The initial daily dose was 400 mg/m^2; the maintenance daily dose was kinetically guided. The maximum value of 500 mg/m^2 should not be exceeded for long without therapeutic monitoring of C_{ss}.

The final analysis has shown the importance of *duration of cytostatic therapy*. The 5-week CHRT seems to be more effective for reaching the target response if compared to the 4-week CHRT because the 5-FU total (cumulative) dose and cumulative AUC were significantly higher (increased by 20–25 %). This fact may partially explain the higher response rate in Group 2 as a consequence of a significantly higher proportion of patients finishing the whole 5-week CHRT (15/20 patients) and better tolerability compared to that registered when using the regimen based on dose escalation schedule (Group 1b).

Pathological Tumour Response to Neoadjuvant Chemotherapy with 5-FU as a Measure of 5-FU Pharmacodynamics: To describe downstaging we have chosen *TNM grading* as this pharmacodynamic end point is considered a significant prognostic factor for patients with stage III rectal cancer and, therefore, is allowed to be taken into account very early when defining the kinetically guided

optimal chemotherapy regimen [37]. Residual disease-free stage (the target pathological response) correlates well with pCR to cytostatic therapy that is examined 2–5 years later [27]. At present, we demonstrate a significant relationship between the cumulative 5-FU AUC and residual disease (%). C_{ss} and AUCs of 5-FU, therefore, seem to be relevant parameters providing information about individual pharmacokinetics of 5-FU, which are predictive for pathological tumour response to neoadjuvant chemotherapy.

Fourteen patients (42 %) who reached the cumulative AUC <50 mg·h/L were undertreated. Sixteen patients reached the cumulative AUC within the range of 50–100 mg·h/L. Only 6 of these 16 patients experienced the target response, while 10 patients experienced only a partial response. Three patients reached AUC >100 mg·h/L and suffered from unacceptable toxicity of CHRT.

Metabolic Ratio: The only significant difference found in the patients who reached the target versus partial response and tolerability was a low metabolic ratio (<2.0) found in the former if compared to the latter. The lower value of the metabolic ratio might account for lower dihydropyrimidine dehydrogenase (DPD) activity. In previous studies a complementary approach based on phenotype was developed, which measures the dihydrouracil/uracil ratio correlating with plasma clearance of 5-FU [15]. We used the dihydrouracil/5-FU ratio, which significantly correlates with residual disease and, therefore, might be considered a phenotyping method effective in identifying some at-risk patients. Used along with kinetically guided dose management, a greater number of at-risk patients can benefit from treatment with the same level of safety and efficacy as non-deficient patients [38]. At-risk patients can continue to be treated with 5-FU with dose adjustments and careful pharmacokinetic follow-up. Our results are consistent with previous ones proving that therapeutic complications such as wide inter- and intraindividual variability in 5-FU kinetics and dynamics/toxicodynamics are at least partially considered a consequence of nongenetic factor influence [39].

Noncompliance with Study Protocol (Therapeutic Scheme): There were three patients (one/Group 1b, two/Group 2) in this study suffering from severe toxicity: enterocolitis (1/3 patients) and perianal dermatitis (2/3 patients). These patients were characterized by very high exposure to 5-FU (cumulative AUC >100 mg·h/L) and a very low metabolic ratio (<1.8) which showed very low plasma concentrations of the inactive metabolite, 5-FUH2. In two patients with perianal dermatitis, the main nongenetic covariate was thought to be their self-medication with folinic acid—noncompliant with the study protocol. This noncompliance was discovered after the study had been terminated. Folinic acid is considered a biochemical modulator (at thymidylate synthase level) dependent on the dose which is often combined with 5-FU in order to enhance the efficacy of cytostatic therapy. Modulations by folinic acid had been identified as independent risk factors by multivariable analysis [39]. Severe toxicity experienced in these two patients can be ascribed to the pharmacodynamic drug–drug interaction manifested at the moment when a higher dose of 5-FU was administered. At present, we have no idea suggesting any nongenetic covariate for severe enterocolitis.

Nevertheless, in these three patients with signs of acute severe toxicity, a low response to CHRT was registered. It might be partly explained by the fact that probable low DPD activity (indicated by the low metabolic ratio) is known as a stronger predictor for toxicity but the role of tumoral activity as a prognostic factor for clinical responsiveness has not been firmly established yet [40]. Moreover, severe toxicity that appeared in these three patients resulted in the prolonged interval between CHRT and the surgery. This interval, which is necessary for recovering after toxic events is also considered a factor predictive for pCR. Unfortunately, this idea was not based on any relevant data [36, 39, 41]. Because of noncompliance with the study protocol, and significantly prolonged interval between CHRT and the surgery, these three patients were excluded from the toxicity analysis.

This event has been demonstrated because patient self-medication might not be rare. This type of noncompliance should be taken into consideration and carefully prevented mainly by patient education.

We noticed one more patient's noncompliance which may also have contributed to therapeutic failure—repeated discontinuation of intravenous infusion for 1–2 h. This fact is considered important because of short 5-FU $t_{½}$ (7–10 min). Approximately 1 h after discontinuation of intravenous infusion, plasma concentration of 5-FU was no longer measurable. Therefore, at the start of therapy every patient should be given written instructions about handling ambulatory chemotherapy with 5-FU.

5 Conclusion

This study demonstrates the linear 5-FU pharmacokinetics (doses versus C_{ss} and AUC) in neoadjuvant chemotherapy of patients with rectal cancer treated with dosing range of 200–500 mg/m^2/24 h with large intra- and interindividual variability in kinetic parameters. Both 5-FU cumulative AUC and 5-FUH2/5-FU ratio are predictive for residual disease of locally advanced rectal cancer. This relationship might be crucial for 5-FU individual dosage adjustment in neoadjuvant CHRT. Provided that intravenous 5-FU continuous infusion is given for 5 weeks with radiotherapy, then the daily dose should exceed 350 mg/m^2 and

cumulative AUC of 5-FU should attain a value within the range of 50–100 mg·h/L. The 5-week CHRT seems to be more effective for reaching the complete response if compared to the 4-week CHRT as the cumulative dose was significantly higher (by 20–25 %). Kinetically guided CHRT based on 5-FU resulted in a higher incidence of target pathological response to therapy and reduced toxicity. Similar predictions should be verified for variable neoadjuvant regimens based on 5-FU.

Acknowledgments We thank Hana Krupičková for excellent technical assistance. This work was supported by a grant from the Ministry of Health (IGA MZ 9357), Czech Republic.

Conflicts of interest of all authors The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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