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ORIGINAL ARTICLE

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Fluoropyrimidine therapy: hyperbilirubinemia as a consequence of hemolysis

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Abstract *Background*: Hemolytic anemia has been noted during treatment with a variety of chemotherapeutic agents. We observed mild compensated hemolytic anemia in a patient receiving capecitabine during a randomized, controlled trial of adjuvant therapy. In order to investigate the hypothesis that hemolysis is the underlying cause of the hyperbilirubinemia sometimes observed during capecitabine treatment, we evaluated factors associated with hemolysis in ten patients. Factors were also analyzed in ten patients receiving 5-fluorourocil/leucovorin (5-FU/LV). *Methods*: Twenty chemotherapy-naïve patients undergoing surgery for Dukes' C colon cancer were included in the phase III, 'X-ACT' trial, and randomized to receive 24-week adjuvant treatment with either oral capecitabine (eight cycles of 1,250 mg/m^2 twice daily for 14 days, followed by a 7-day rest period) ($n=10$) or 5-FU/LV administered according to the Mayo Clinic regimen (six cycles of LV 20 mg/m^2 followed by 5-FU 425 mg/m^2, administered as an i.v. bolus on days 1–5 every 28 days) ($n=10$). Ten patients randomized in each treatment arm were evaluated. Hemolytic parameters evaluated included bilirubin, lactate dehydrogenase, haptoglobin, and reticulocytes. *Results*: Seven patients receiving capecitabine and three patients receiving 5-FU/LV experienced grade 1/2 elevations of bilirubin during the 24-week treatment period. In most cases, hyperbilirubinemia was associated with concomitant alterations in other hemolytic parameters. Five episodes of grade 1 compensated hemolytic anemia were reported in four capecitabine-treated patients, all of which were associated with hyperbilirubinemia. *Conclusion*: Adjuvant treatment with capecitabine or 5-FU/LV in a small sample of patients with Dukes' C colon cancer was associated with alterations in hemolytic parameters. These alterations, in particular hyperbilirubinemia, were associated in some patients with low-grade compensated hemolytic anemia. All changes were clinically insignificant, fully reversible, and may represent a fluoropyrimidine class effect. Further studies are indicated to evaluate the incidence and implications of this effect.

Keywords 5-FU · Capecitabine · Hemolysis · Bilirubin · Colon cancer · Adjuvant

Abbreviations *5-FU*: 5-Fluorouracil · *CI*: Confidence interval · *CTC*: Common toxicity criteria · *CTCAE*: Common terminology criteria for adverse events · *DFS*: Disease-free survival · *i.v.*: Intravenous · *KPS*: Karnofsky performance score · *LDH*: Lactate dehydrogenase · *LV*: Leucovorin · *MCV*: Mean corpuscular volume · *NCI*: US National Cancer Institute · *NCIC*: National Cancer Institute of Canada · *TIBC*: Total iron binding capacity · *UFT*: Uracil plus tegafur · *ULN*: Upper limit of normal

Introduction

Anemia is a frequent complication of malignancy. Most anemias observed are of the chronic and hypoproliferative type termed 'the anemia of chronic disease'. However, hemolytic anemia has also been noted in association with some hematological malignancies and solid tumors. It has become apparent that hemolytic anemia may also occur as a result of the administration of chemotherapeutic agents, including platinum-based compounds, mitomycin, cyclophosphamide, and fluoropyrimidines [9, 12].

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Fluoropyrimidine compounds, with 5-fluorouracil (5-FU) as their principal representative, have been a mainstay of chemotherapy for solid tumors for over 40 years. 5-FU, either as monotherapy or in combination with other cytotoxic agents or biomodulators, is used in many standard treatment regimens for common solid tumors, including breast and colorectal cancers. Schedule modifications and biomodulation have improved the efficacy and tolerability of 5-FU [1–3, 5, 7, 14, 16, 18], but complications associated with infused intravenous (i.v.) administration and patient preference for oral agents [4, 15] have driven the development of oral fluoropyrimidines including capecitabine, uracil plus tegafur (UFT), and eniluracil.

Two large, multicenter, phase III clinical trials have shown that the novel oral fluoropyrimidine, capecitabine, is at least as effective as i.v. 5-FU in the first-line treatment of metastatic colorectal cancer [11, 23, 24]. In these two trials, capecitabine achieved significantly superior response rates and equivalent time to disease progression and overall survival compared with i.v. bolus 5-FU/leucovorin (LV) (Mayo Clinic regimen). As first-line therapy for metastatic colorectal cancer, capecitabine also demonstrated an improved safety profile compared with 5-FU/LV, with significantly lower incidences of diarrhea, stomatitis, nausea, alopecia, and grade 3/4 neutropenia, leading to significantly fewer cases of neutropenic fever/sepsis and associated hospitalizations [6]. The only side effects occurring more frequently with capecitabine were the cutaneous condition, hand-foot syndrome, and the laboratory abnormality, hyperbilirubinemia. Grade 3 hyperbilirubinemia occurred in 18% of patients treated with capecitabine versus 3% of patients who received i.v. 5-FU/LV. However, the incidence of grade 4 hyperbilirubinemia was low in both treatment groups (4.5 vs. 2.5%, respectively). In these trials, side effects were graded according to the National Cancer Institute of Canada (NCIC) common toxicity criteria (CTC) scale (revised 1991) [6]. With this system, the severity of hyperbilirubinemia is graded differently to the more current US National Cancer Institute (NCI) CTC (version 2.0) and Common Terminology Criteria for Adverse Events (CTCAE; version 3.0) scales (Table 1). Hyperbilirubinemia classified as grade 3 in severity by the NCIC scale corresponds to grade 2 in the newer NCI scales. Similarly, grade 4 severity according to the NCIC scale [except in the case of values $> 10 \times$ upper limit of normal (ULN)] corresponds to grade 3 in the NCI scales. With capecitabine, hyperbilirubinemia tended to be an isolated laboratory abnormality and was rarely associated with hepatobiliary or other abnormalities. Furthermore, baseline elevations in liver function parameters, including elevations in transaminase or alkaline phosphatase concentrations, did not correlate with hyperbilirubinemia during treatment with capecitabine [6].

Hyperbilirubinemia has also been observed in other clinical trials that compared other oral fluoropyrimidines with i.v. 5-FU. In a phase III, comparative trial of oral eniluracil/5-FU versus i.v. bolus 5-FU/LV (Mayo Clinic regimen), hyperbilirubinemia (all grades) was noted in 41% of patients who received eniluracil/5-FU and 20% of those who received 5-FU/LV [21]. The incidence of grade 3/4 hyperbilirubinaemia was 22% and 9%, respectively. In another phase III study of oral UFT plus LV versus i.v. bolus 5-FU/LV, hyperbilirubinemia (any grade) was observed in 44% of patients in the UFT/LV treatment group and 22% of those in the 5-FU/LV group, with grade 3/4 hyperbilirubinemia occurring in 15% and 8% of patients, respectively [10]. These observations suggest that the hyperbilirubinemia associated with fluoropyrimidines may be a class effect. This hyperbilirubinemia does not appear to impact significantly on liver function, or to be associated with an adverse clinical outcome. Isolated hyperbilirubinemia, in conjunction with elevated levels of lactate dehydrogenase (LDH) but normal hepatocellular enzymes, is typically observed during hemolysis. Hemolysis may frequently be subclinical, but if marked, may present clinically as severe anemia, with typical symptoms of fatigue, weakness, and shortness of breath on exertion.

A large international, randomized, phase III trial (X-ACT) has compared 6-months' treatment with adjuvant capecitabine or i.v. 5-FU/LV (Mayo Clinic regimen) in 1987 patients following surgery for Dukes' C colon cancer. The primary endpoint of the trial—to demonstrate the equivalence of both treatments in terms of disease-free survival (DFS)—was clearly met [hazard ratio: 0.87; 95% confidence interval (CI): 0.75–1.00, $P < 0.001$ vs. upper CI limit of 1.20] [22]. Moreover, capecitabine treatment showed a strong trend toward superior DFS ($P = 0.0528$), resulting in a 3.6% improvement at 3 years compared with 5-FU/LV. Adjuvant capecitabine was also associated with significantly

Table 1 Hyperbilirubinemia grading: NCIC and NCI scales

Grade of bilirubin elevation	NCIC (revised 1991)	NCI CTC (version 2.0; 1999) NCI CTCAE (version 3.0; 2003)
0	WNL	WNL
1		> ULN – 1.5 × ULN
2	> WNL – < 1.5 × ULN	> 1.5 – 3.0 × ULN
3	1.5 – 3.0 × ULN	> 3.0 – 10.0 × ULN
4	> 3.0 × ULN	> 10.0 × ULN

CTC Common toxicity criteria, *CTCAE* Common terminology criteria for adverse events, *NCI* National Cancer Institute, *NCIC* National Cancer Institute of Canada, *ULN* upper limit of normal, *WNL* within normal limit

superior relapse-free survival (hazard ratio: 0.86; $P = 0.0407$) and showed a trend toward superior overall survival (hazard ratio: 0.84; $P = 0.0706$) versus 5-FU/LV. Similar to trials conducted in the metastatic setting [6], the NCIC CTC scale was used for the grading of adverse events. In this trial, grade 3/4 hyperbilirubinemia was observed more frequently with capecitabine compared with 5-FU/LV (20% vs. 6%, respectively) [20]. However, it should be noted that if the more current NCI grading systems were used, the incidence of grade 3/4 hyperbilirubinemia would be negligible in both treatment arms (1.4 vs. 0.3%, respectively).

In the first patient enrolled into the X-ACT trial at the Belgrade center, we observed mild compensated hemolytic anemia, which we hypothesized could potentially be the underlying cause of hyperbilirubinemia associated with capecitabine treatment. Based on this observation, we decided to prospectively evaluate factors associated with hemolysis, in addition to the standard protocol-defined laboratory parameters, in these chemotherapy-naïve patients who were receiving capecitabine as adjuvant therapy following surgery for Dukes' C colon cancer. To investigate the hypothesis that hemolysis may represent a fluoropyrimidine class effect, hemolytic factors were also analyzed in patients treated with adjuvant 5-FU/LV at the same center within the same clinical trial. However, as it was not possible to apply the same schedule of assessments to patients receiving 5-FU/LV and capecitabine, comparative analyses could not be performed.

Methods

Patients and treatment

Twenty patients were enrolled into the X-ACT trial at the Belgrade site; all were chemotherapy naïve, and had undergone surgery with curative intent for Dukes' C colon cancer. Ten patients were randomized to receive adjuvant therapy with oral capecitabine (eight cycles of 1,250 mg/m^2 twice daily for 14 days, followed by a 7-day rest period). A further ten patients received 5-FU/LV administered according to the Mayo Clinic regimen (six cycles of LV 20 mg/m^2 followed by 5-FU 425 mg/m^2, administered as an i.v. bolus on days 1–5 every 28 days). Hemolytic factors were evaluated in all 20 patients. The supplementary blood sampling required additional witnessed oral consent of the chosen patients, and was conducted in accordance with the local ethics committee. The analyses reported here are exploratory, as the study was not intended or adequately powered for comparative analyses.

Assessment of safety and laboratory parameters

Safety was evaluated in all patients, and for these analyses, adverse events were graded according to the NCI CTC scale (version 2.0) (Table 1).

Protocol-specified laboratory parameters were evaluated in patients before every treatment cycle (i.e. every 3 or 4 weeks depending on the treatment arm), and included: total white and red blood cell counts; differential white blood cell counts; hemoglobin; platelet counts; hematocrit; total bilirubin (with conjugated [direct] bilirubin assessed in the case of abnormal total bilirubin); alanine transaminase; aspartate transaminase; γ-glutamyl transferase; albumin or total protein; serum creatinine; uric acid; electrolytes; serum calcium; and inorganic phosphate. In addition, the following parameters were evaluated (normal ranges indicated in parentheses): mean corpuscular volume (MCV) (80.0–99.9 fl/cell); serum iron (male: 11–28 μM, female: 7–20 μM); total iron binding capacity (TIBC) (46.4–68.5 μM); haptoglobin (≥0.450 g/l); reticulocytes (≤ 4%); LDH (230–460 U/l); and direct Coombs' test (negative).

Blood samples were obtained from patients according to the following schedules. In patients receiving capecitabine, blood samples were obtained weekly throughout treatment from day 1 (before treatment) to day 168 (the last day of the 24-week treatment period). As no comparative analyses were intended, for practical reasons, blood samples were obtained on day 1 (before treatment), at the end of every 2 weeks from weeks 2–8, and then at the end of every 4 weeks until the end of treatment in patients receiving the 4-weekly 5-FU/LV regimen (Fig. 1). In both treatment groups, blood samples were also obtained after the end of the treatment period, during patient follow-up.

Peripheral blood counts, including reticulocytes, were determined using an automated analyzer (CELL-DYN 3.500, Abbott Laboratories, Chicago, USA; according to the manufacturer's instructions). Serum iron, TIBC,

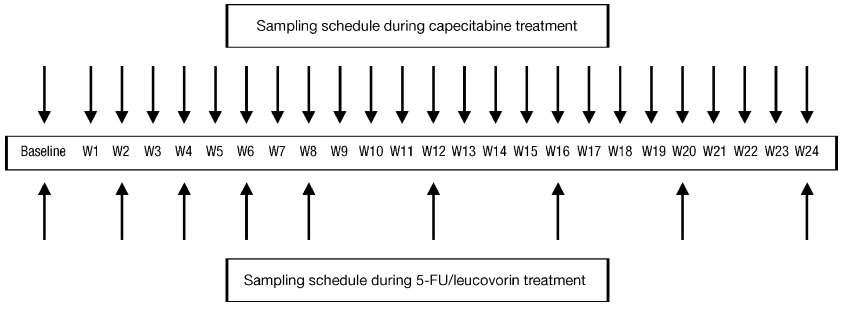

Fig. 1 Laboratory sampling schedule during treatment with capecitabine and 5-FU/LV

total and direct bilirubin were assayed using an automated analyzer (Spectrum, Abbott Laboratories, Chicago, USA; according to the manufacturer's instructions). Erythrocytes, obtained from citrated plasma, were used for the direct Coombs' test, which was conducted according to Dausset's method using antihuman globulin and anti-IgG and anti-C3 monospecific antihuman sera (Dade-Behring, Marburg GmBH, Marburg, Germany; according to the manufacturer's instructions).

Results

Patients and their clinical evolution

As in the overall trial population [22], baseline characteristics were similar in both groups of patients analyzed here. The median age of patients was 52 years (range 35–69) in the capecitabine group and 60 years (range 39–72) in the 5-FU/LV group. The median time from surgery to randomization was 39 days in both groups. All patients randomized to receive capecitabine had a Karnofsky Performance Score (KPS) of 100% at baseline. In the 5-FU/LV group, eight patients had a baseline KPS of 100%, and two had a baseline KPS of 90%.

Overall safety profile

Both treatments were generally well tolerated by the patients. None of the patients who received capecitabine or 5-FU/LV withdrew from treatment due to treatment-related adverse events. Four patients who received capecitabine experienced grade 1 compensated hemolytic anemia. Other adverse events (related or unrelated to treatment) experienced by the patients who received capecitabine were thrombophlebitis not requiring anticoagulant therapy (two patients), and a transient loss of consciousness during administration of i.v. contrast for computed tomography (CT) scanning (one patient). Adverse events occurring in the 5-FU/LV group included diarrhea (two patients [grade 1 and grade 4 intensity, respectively]), and stomatitis (one patient [grade 2])

Laboratory signs of hemolysis

Changes in bilirubin levels

Table 2 shows the highest total and direct bilirubin concentrations and corresponding grade of hyperbilirubinemia (according to the NCI CTC scale [version 2.0] [Table 1]) experienced by patients in each treatment group during the 24-week treatment period. No patients in either treatment group had bilirubin levels outside the normal range at study entry. Grade 1/2—but no grade 3/4—bilirubin elevations occurred in both treatment groups.

In patients with hyperbilirubinemia, bilirubin remained predominantly in the unconjugated form, with only three patients in the capecitabine treatment arm and two in the 5-FU/LV arm having direct bilirubin levels above 10 μM (normal range <7 μM). An additional two patients who received capecitabine and one who received 5-FU/LV had very mild elevations in direct bilirubin (range 7–10 μM). Tables 3 and 4 show the mean levels of bilirubin at different time points in the assessment schedule for patients who received capecitabine and 5-FU/LV, respectively.

Changes in LDH levels

There were no significant changes in mean LDH levels in either the capecitabine or the 5-FU/LV groups (Tables 3, 4). Peak LDH levels in the capecitabine group (468 ± 158 U/L) were noted after 14 weeks of treatment

Table 2 Maximum bilirubin concentrations (C_{max}) and worst grade of hyperbilirubinemia observed in each patient during the 24-week treatment period

Patient Number	Capecitabine				5-FU/LV			
	Total bilirubin		Direct bilirubin (μM)	Day of treatment	Total bilirubin		Direct bilirubin (μM)	Day of treatment
	C_{max} (μM)	Grade[a]			C_{max} (μM)	Grade[a]		
1	43.6	2	8.2	77 (cycle 4)[b]	45.9	2	12.3	112 (cycle 4)
2	13.9	0	3.8	–	14.1	0	5.1	–
3	17.1	0	4.9	–	13.3	0	2.9	–
4	38.8	2	26.4	147 (cycle 7)[b]	7.6	0	4.9	–
5	16.2	0	5.4	–	10.1	0	0.9	–
6	30.5	1	5.0	133 (cycle 7)	35.9	2	12.3	43 (cycle 2)
7	37.7	2	8.7	63 (cycle 3)[b]	10.8	0	2.8	–
8	41.3	2	15.3	155 (cycle 8)	27.6	1	8.6	168 (cycle 6)
9	30.7	1	6.9	126 (cycle 6)[c]	12.5	0	2.7	–
10	36.4	2	10.3	99 (cycle 5)	9.2	0	2.8	–

[a]According to National Cancer Institute Common Toxicity Criteria
[b]Coinciding with an episode of hemolytic anemia
[c]Patient experienced hemolytic anemia on days 63–71 of treatment

Table 3 Mean values for hemolytic parameters in the capecitabine group ($n = 10$)

	Parameter, mean value ± standard deviation							
	Total bilirubin (μM)	Direct bilirubin (μM)	LDH (U/l)	Haptoglobin (g/l)	Reticulocytes (%)	Hemoglobin (g/dl)	MCV fl/cell	Iron (μM)
Baseline	10.2 ± 2.8	3.4 ± 1.8	361 ± 113	2.4 ± 1.2	2.5 ± 1.5	116.1 ± 14.4	82.0 ± 7.6	12.3 ± 4.4
Days 14–15	12.3 ± 6.7	4.2 ± 3.3	371 ± 75	1.4 ± 1.2	2.9 ± 1.5	116.8 ± 12.5	82.8 ± 7.7	16.0 ± 6.5
Days 28–29	12.1 ± 4.2	2.7 ± 0.6	361 ± 88	1.8 ± 1.4	1.9 ± 0.5	117.1 ± 15.5	86.7 ± 5.3	20.2 ± 8.5
Days 42–43	14.9 ± 6.9	4.3 ± 1.7	376 ± 59	1.6 ± 1.2	4.0 ± 1.4	117.4 ± 13.7	86.6 ± 7.1	10.3 ± 3.3
Days 56–57	19.4 ± 8.9	5.4 ± 2.1	468 ± 158	1.0 ± 1.1	3.5 ± 1.6	119.9 ± 11.9	88.3 ± 7.0	23.9 ± 9.0
Days 84–85	17.9 ± 8.4	4.6 ± 2.5	430 ± 128	1.3 ± 1.2	5.7 ± 1.9	120.2 ± 12.6	91.9 ± 7.4	14.2 ± 7.2
Days 112–113	18.4 ± 6.8	4.8 ± 2.6	390 ± 78	1.3 ± 1.2	2.6 ± 1.0	121.3 ± 11.2	94.6 ± 7.9	21.0 ± 10.8
Days 140–141	18.4 ± 8.0	8.0 ± 5.6	438 ± 107	1.5 ± 1.5	2.5 ± 1.1	124.2 ± 12.5	96.4 ± 8.9	23.1 ± 12.7
Post-treatment (week 24–27)	16.4 ± 6.0	3.9 ± 3.0	404 ± 140	0.9 ± 0.6	3.0 ± 1.9	122.8 ± 12.6	95.8 ± 9.5	11.8 ± 6.6

LDH lactate dehydrogenase, *MCV* mean corpuscular volume

Table 4 Mean values for hemolytic parameters in the 5-FU/LV group ($n = 10$)

	Parameter, mean value ± standard deviation							
	Total bilirubin (μM)	Direct bilirubin (μM)	LDH (U/l)	Haptoglobin (g/l)	Reticulocytes (%)	Hemoglobin (g/dl)	MCV fl/cell	Iron (μM)
Baseline	9.6 ± 1.7	2.2 ± 0.5	345 ± 47	3.4 ± 1.6	2.2 ± 0.7	109.6 ± 11.8	80.5 ± 5.7	11.6 ± 10.6
Days 14–15	10.1 ± 3.9	2.9 ± 1.1	314 ± 37	3.2 ± 1.8	2.6 ± 1.4	109.8 ± 10.3	81.1 ± 5.9	8.5 ± 5.3
Days 28–29	9.9 ± 4.3	2.7 ± 0.8	326 ± 43	2.7 ± 1.6	2.4 ± 1.4	113.5 ± 11.7	82.5 ± 6.3	9.8 ± 4.5
Days 42–43	15.4 ± 9.5	4.1 ± 3.4	362 ± 80	2.7 ± 1.6	3.1 ± 1.6	113.8 ± 9.3	82.2 ± 6.3	9.7 ± 6.1
Days 56–57	11.6 ± 5.0	3.3 ± 1.5	373 ± 101	2.5 ± 2.0	2.6 ± 0.9	117.1 ± 10.9	84.7 ± 5.9	11.5 ± 5.2
Days 84–85	11.7 ± 4.9	3.4 ± 1.7	373 ± 105	2.0 ± 1.6	2.4 ± 1.0	116.7 ± 11.9	85.7 ± 6.8	12.2 ± 5.6
Days 112–113	14.8 ± 12.2	4.3 ± 3.4	352 ± 99	2.2 ± 1.3	2.9 ± 1.3	117.7 ± 12.8	87.2 ± 7.1	12.2 ± 5.4
Days 140–141	11.7 ± 6.2	3.3 ± 2.5	366 ± 32	2.3 ± 1.6	2.0 ± 0.9	116.7 ± 11.9	88.2 ± 7.8	10.8 ± 5.4
Post-treatment (week 24–27)	12.8 ± 7.9	3.1 ± 2.5	355 ± 49	1.8 ± 1.2	2.6 ± 1.3	117.7 ± 12.8	89.2 ± 8.2	10.4 ± 4.3

LDH lactate dehydrogenase, *MCV* mean corpuscular volume

(Table 3). This is the same time at which peak mean bilirubin concentrations (19.4 ± 8.9 U/L) were noted (Table3). All patients with hyperbilirubinemia had elevated LDH during treatment. A direct association between bilirubin and LDH levels was noted in six patients who received capecitabine and one patient who received 5-FU/LV (data not shown). An example of this association in one capecitabine-treated patient is shown in Fig. 2.

Changes in haptoglobin levels

In capecitabine-treated patients, haptoglobin decreased from a mean value of 2.4 ± 1.2 g/l at baseline to a minimum of 1.0 ± 1.1 g/l after 8 weeks of treatment (Table3). During treatment with 5-FU/LV, mean haptoglobin decreased from 3.4 ± 1.6 g/l at baseline to a minimum of 2.0 ± 1.6 g/l after 12 weeks of treatment (Table4). An association between decreasing haptoglobin and increasing bilirubin was noted in four patients who received capecitabine and one patient who received 5-FU/LV (data not shown). An example of the relative changes in haptoglobin and bilirubin in one capecitabine-treated patient is shown in Fig. 3.

Changes in reticulocyte levels

Mean reticulocyte levels increased significantly in patients who received capecitabine, from a baseline level of 2.4 ± 1.5% to a peak of 5.7 ± 1.9% after 12 weeks of treatment (Table 3). In the group of patients who received 5-FU/LV, reticulocyte levels increased from a baseline mean of 2.2 ± 0.7% to 3.1 ± 1.6% after 6 weeks of therapy (Table 4). Concomitant increases in reticulocytes were associated with increased bilirubin in five patients who received capecitabine (example in Fig. 4) and one patient who received 5-FU/LV (data not shown).

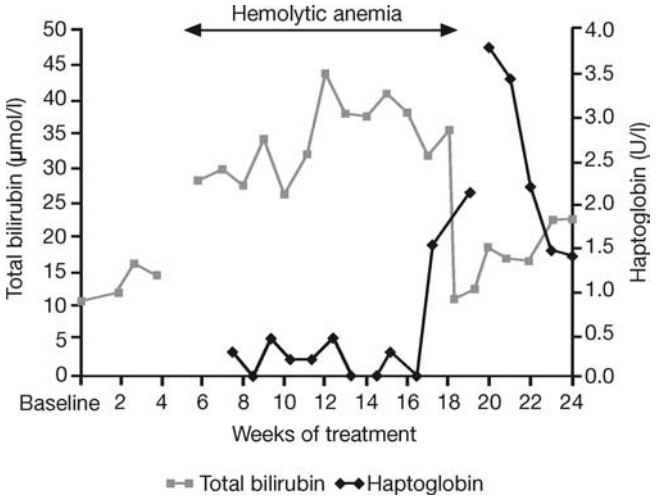

Fig. 3 Changes in bilirubin and haptoglobin levels during 24 weeks of treatment with capecitabine

Relation between hemolysis and hyperbilirubinemia

Four capecitabine-treated patients experienced episodes of grade 1 compensated hemolytic anemia during the study. The criteria for hemolytic anemia were: decreased hemoglobin, indirect hyperbilirubinemia, reticulocytosis, decreased haptoglobin levels, and increased LDH. The characteristics of these episodes of hemolytic anemia, together with key laboratory abnormalities observed in association with these episodes, are summarized in Table 5. One patient experienced two protracted episodes of mild hemolytic anemia. The first of these episodes developed on day 14 of cycle two and resolved at the end of cycle six; the second developed on day 14 of the last treatment cycle (cycle eight) and resolved 55 days following the end of treatment. This patient yielded a negative direct Coombs' test at

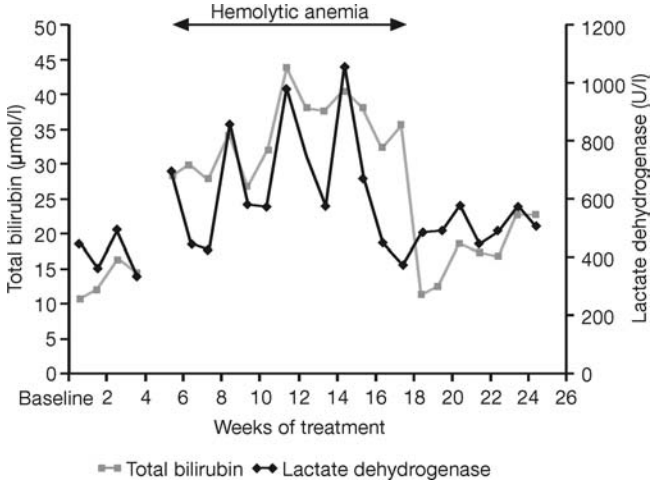

Fig. 2 Changes in bilirubin and LDH levels during 24 weeks of treatment with capecitabine

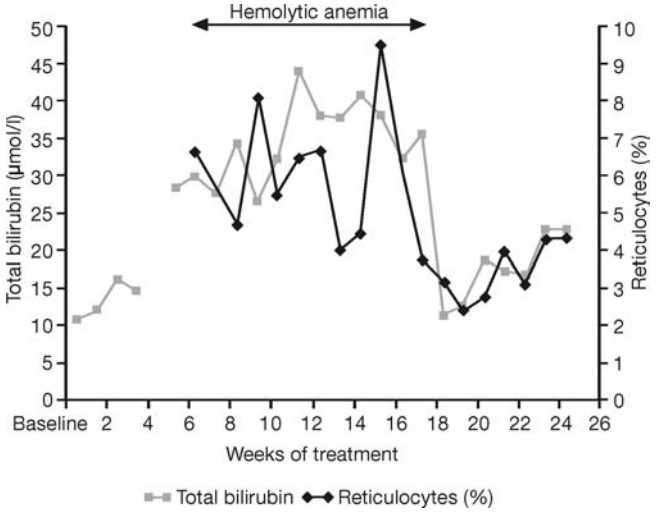

Fig. 4 Changes in bilirubin and reticulocyte levels during 24 weeks of treatment with capecitabine

Table 5 Characteristics of hemolytic anemia in patients receiving capecitabine: onset, duration, and key laboratory abnormalities (most abnormal concentration associated with hemolytic anemia is shown)

Patient no.	1 [2351]		7 [2359]	9 [2360]	4 [2369]
	Episode 1	Episode 2			
Day of onset	35	161	57	63	147
Duration (days)	91	64	14	8	3
Direct Coombs' test	Negative	Negative	Negative	Negative	ND
Abnormal laboratory parameters (normal range)					
Total bilirubin (3.4–20.5 μM)	43.6	28.0	37.7	21.9	38.8
Direct bilirubin (0–6.85 μM)	9.2	–	8.8	–	26.4
LDH (230–460 U/l)	1060	736	504	506	639
MCV (80.0–99.9 fl/cell)	–	–	–	–	104.1
Reticulocytes (≤ 4%)	9.5	4.3	–	6.2	–
TIBC (46.4–68.5 μM)	79.4	76.0	71.6	80.4	121.2
Hemoglobin (130–170 g/dl)[a]	102	108	–	100	142
Haptoglobin (≥0.450 g/l)	0	1.38	–	0.19	ND
Serum iron (11–28 μM)[a]	5.4	6.5	1.4	9.4	62.0

LDH lactate dehydrogenase, *MCV* mean corpuscular volume, *ND* not determined, *TIBC* total iron binding capacity
[a] Normal range for males; all patients developing hemolytic anemia were male

baseline, and on days 43, 49, 56, and 185 following the start of treatment. The other three patients experienced single episodes of hemolytic anemia, which lasted less than 14 days and developed during cycle two in two patients (on days 15 and 21 of the drug-free period, respectively), and at the end of cycle seven in the remaining patient. The direct Coombs' test was negative at baseline and following the end of the treatment (on days 170 and 177, respectively) in the two patients from whom samples were tested. Hyperbilirubinemia was present during all five episodes (in four patients) of compensated hemolytic anemia (Table 5).

No episodes of compensated hemolytic anemia were noted in patients who received 5-FU/LV. One patient in this group had moderate hyperbilirubinemia during the fourth cycle of treatment. This patient showed an association between bilirubin, LDH, reticulocyte, and haptoglobin levels (data not shown). Two further 5-FU/LV-treated patients were noted to have elevated bilirubin levels during the study.

The observed changes in bilirubin, LDH, haptoglobin, and reticulocytes (Tables 3, 4; Figs. 2, 3, 4), along with the episodes of hemolytic anemia (Table 5), suggest that the hyperbilirubinemia observed in a number of patients may occur as a result of hemolysis.

Changes in other hematologic parameters

In both groups of patients, mean hemoglobin levels remained relatively constant during treatment (Tables 3, 4). The majority of patients were mildly anemic at study entry, a typical observation in a colorectal cancer population. Increases in MCV over the course of the study were observed in both treatment groups (Fig. 5). The effect of treatment on MCV was reversible, with mean volumes decreasing following the end of treatment (Fig. 5).

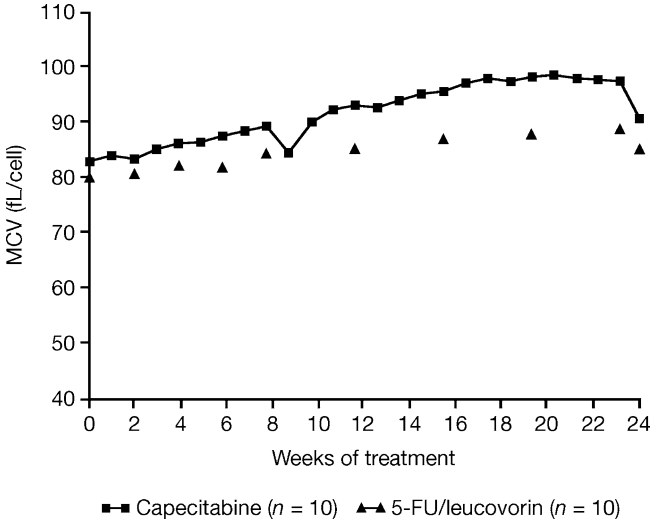

Fig. 5 Impact of treatment on mean MCV during treatment with capecitabine and 5-FU/LV

Discussion

Analysis of the two groups showed treatment with both capecitabine and 5-FU/LV to be associated with time-dependent alterations in hemolytic parameters, including bilirubin, LDH, and haptoglobin. These changes were clinically insignificant and reversible, with all parameters returning to baseline after discontinuation of chemotherapy. In four capecitabine-treated patients, changes in hemolytic parameters, in particular hyperbilirubinemia, were associated with sub-clinical compensated hemolytic anemia. The considerable difference in sampling frequency in the capecitabine arm compared with the 5-FU/LV arm—due to differences in treatment cycle length and blood sampling times—precluded any

comparison of changes occurring in the two treatment groups. The most comprehensive data are available for the capecitabine-treated patients, from whom weekly samples were obtained. In the 5-FU/LV group, the majority of samples were collected before the start of each treatment cycle (i.e. 23 days after the last dose of 5-FU/LV).

No grade 3/4 elevations in bilirubin occurred in either group. Four of the seven patients who experienced hyperbilirubinemia in the capecitabine group also experienced grade 1 hemolytic anemia. These four cases of hemolytic anemia were all mild, and a direct Coombs' test was negative in each of the three cases tested, suggesting no association with immune causes. Increases in MCV over the course of the study occurred in both treatment arms, which may indicate that both treatments accelerate the rate of erythropoeisis, potentially as a result of treatment-related hemolysis. Increases in MCV without concomitant anemia have been observed recently in patients receiving capecitabine [13]. In a retrospective review of 76 consecutive patients with metastatic breast cancer who received standard 21-day cycles of oral capecitabine, MCV was found to increase in a dose-dependent and time-dependent manner, with 57% of patients developing macrocytosis (MCV $>$ 100 fl) [13]. Development of macrocytosis was shown to be independent of anemia, thrombocytopenia, neutropenia, liver metastasis, and hepatic dysfunction. In another study, 154 patients with various malignancies received 21-day cycles of capecitabine as either monotherapy or in combination with other antineoplastic agents [25]. A significant increase in MCV compared with baseline was observed within 9 weeks of treatment, in the absence of any other hematologic abnormalities or clinical symptoms. The increase in MCV was higher in patients with tumor remission or stable disease compared with patients with tumor progression. The authors of this study hypothesized that an increase in MCV might occur as a result of 5-FU-mediated inhibition of thymidylate synthesis, leading to slow formation of erythroid cell DNA [25].

Due to the small number of patients studied, and the collection of fewer samples during the active treatment phase in the 5-FU/LV group, definitive conclusions about the impact of 5-FU/LV treatment on hemolytic parameters cannot be drawn. Results suggest that treatment with 5-FU/LV is also associated with alterations in hemolytic parameters. These findings, in conjunction with those observed in other clinical trials of oral fluoropyrimidines and i.v. 5-FU [12, 6, 10, 21], imply that alterations in hemolytic parameters may represent a fluoropyrimidine class effect. However, the frequency of hemolytic abnormalities appears to be less with 5-FU/LV than with the oral fluoropyrimidines, capecitabine, UFT and eniluracil. This may be due to differences in the metabolism of oral agents and 5-FU/LV, or alternatively, may be a dose/schedule-related phenomenon. Oral fluoropyrimidines enable chronic dosing that provides more prolonged exposure to 5-FU compared with bolus i.v. 5-FU/LV.

Several case reports of hemolytic anemia associated with the administration of 5-FU [12, 19] and UFT [26] have been reported in the literature. One of these publications by Zurita Saavedra et al. [26] reported a case of hemolytic anemia in a patient who received UFT for metastatic colon cancer. The authors noted that the temporal relationship between UFT intake and the appearance of the hemolytic anemia, and the rapid recovery and lack of relapse after the drug was stopped, was suggestive of a causal relationship. Interestingly, hemolytic anemia has also been associated with administration of a number of other cytotoxic agents including oxaliplatin [8], carboplatin [17], cisplatin, methotrexate, melphalan, and doxorubicin [9]. Physicians should always consider the possibility of drug-induced hemolytic anemia in any patient who has a sudden or unexplained drop in hemoglobin concentration while undergoing chemotherapy.

In conclusion, our findings add to the small body of literature describing hemolytic anemia in patients receiving cytotoxic therapy. This small pilot study generates the hypothesis that hemolysis might be the mechanism potentially underlying the development of hyperbilirubinemia in patients receiving fluoropyrimidines. The observed alterations in hemolytic parameters suggest that fluoropyrimidines may exert a class effect that is fully reversible after cessation of therapy. Considering a previous hypothesis that the increase in MCV is due to 5-FU-mediated inhibition of thymidylate synthetase in erythroid cells (leading to inhibition of DNA synthesis) [25], we speculate that hemolysis may occur because of defective formation of the red cell membrane that results from fluoropyrimidine-mediated thymidylate synthetase inhibition in erythroblasts. However, further studies are warranted to clarify the mechanism of hemolysis in patients treated with fluoropyrimidines.

For patients with colorectal cancer, capecitabine offers consistent efficacy and safety benefits over i.v. 5-FU/LV, in both the metastatic and adjuvant settings. Capecitabine also offers patients a more convenient treatment option that can be administered at home, and which avoids the medical complications and psychological distress associated with venous access. As the oral agents are now enabling new adjuvant strategies and long-term maintenance chemotherapy, the examination of their impact on hemolytic parameters and possible clinical implications of hemolysis is becoming increasingly important.

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