Capecitabine treatment results in increased mean corpuscular volume of red blood cells in patients with advanced solid malignancies

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Capecitabine is a novel fluoropyrimidine carbamate which is selectively activated after oral administration to 5-fluorouracil (5-FU) by a sequential triple enzyme pathway in liver and tumor cells. The cytotoxic activity of the metabolized 5-FU depends on thymidylate synthase (TS) inhibition, leading to defective DNA synthesis. Capecitabine has shown promising activity in all tumor types sensitive to 5-FU and is therefore investigated in many clinical trials. Since we observed an increase of mean corpuscular volume (MCV) of red blood cells under therapy with capecitabine, the current investigation aimed to quantitate this effect and to elucidate the underlying mechanisms. A total of 154 patients suffering from advanced cancer received capecitabine (2500 mg/m^2/day for 14 days every 21 days) either as monotherapy, or in combination with other antineoplastic agents or biological response modifiers. During 3 consecutive cycles of therapy a complete blood cell count including the red cell indices MCV, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration was performed before each application of capecitabine. In addition, vitamin B_{12}, folic acid and homocysteine were determined to define their role in increasing MCV. Restaging was performed after 9 weeks. Within 9 weeks, a statistically significant increase of MCV (without other hematologic abnormalities or clinical symptoms) could be observed ($p < 0.0001$). Vitamin B_{12}, folic acid and homocysteine levels did not change significantly during the observation period. When comparing the different increases of MCV during 9 weeks (ΔMCV) with respect to tumor response, ΔMCV tended to higher values in patients with tumor remission or stable disease than in patients with tumor progression. We conclude that serum levels within the normal range rule out severe deficiencies of vitamin B_{12}, folic acid or homocysteine as an account of macrocytemia. We therefore hypothesize that an increased MCV (without concomitant anemia) in patients receiving capecitabine might be due to the 5-FU-induced TS inhibition also in erythroid precursor cells. Whether this increase in MCV might serve as a surrogate marker for tumor response has to be evaluated in further investigations. *Anti-Cancer Drugs* 14:119–123 © 2003 Lippincott Williams & Wilkins.

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Introduction

For more than 40 years the fluoropyrimidines, with 5-fluorouracil (5-FU) as their most important representative, have been included in the standard treatment for a wide range of common solid tumors. In addition to its leading role in colorectal cancer [1], 5-FU, administered either as monotherapy or in combination with other cytotoxic agents or biomodulators, shows substantial benefits in a number of tumor entities such as breast cancer or colorectal cancer [2–4]. The cytotoxic action of 5-FU is mostly based on the inhibition of thymidylate synthase (TS). This effect is mediated by the 5-FU metabolite 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), which blocks the *de novo* synthesis of thymidylate (dTMP), an essential enzyme for DNA synthesis, by forming a ternary complex with TS and the essential co-factor 5,10-methylenetetrahydrofolate (CH_2-THF) [5,6].

However, the quick fall of plasma concentrations below the cytotoxic threshold (due to rapid degradation) limits the therapeutic effects of i.v. bolus 5-FU. This effect may be overcome by applying capecitabine (n^4-pentoxylcarbonyl-5′-deoxy-5-fluorocytidine, Xeloda; Hoffman-La Roche, Nutley, NJ), an orally administered fluoropyrimidine carbamate, which is activated by a three-step enzymatic conversion to the actual antineoplastic agent 5-FU. After ingestion, capecitabine is rapidly absorbed as an intact molecule. In the first step, capecitabine is hydrolyzed by carboxylesterase in the liver to 5′-deoxy-5-

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fluorocytidine (5′-DFCR). In the next step, cytidine deaminase, which occurs in the liver and/or tumor tissue, converts 5′-DFCR to 5′-deoxy-5-fluorouridine (5′-DFUR) [7,8]. The last step occurs at the tumor site by the tumor-associated angiogenic factor thymidine phosphorylase (TP), metabolizing 5′-DFUR to 5-FU [7,9,10]. Therefore, 5-FU is preferentially generated in tumor tissue when compared with normal body tissue.

In consequence, many clinical trials are investigating the role of capecitabine as treatment for different tumor entities [11], with promising results published [12–15]. Usually, treatment with capecitabine is well tolerated with only moderate side effects including nausea, diarrhea, fatigue, the hand–foot syndrome and laboratory abnormalities such as myelosuppression or increases in serum bilirubin [12–15].

When dealing with new drugs, the clinician thoroughly monitors side effects and thus checks carefully the routinely evaluated laboratory parameters with special attention to the complete blood count. As we did in patients receiving oral therapy with capecitabine, we interestingly did not note neutropenia or thrombocytopenia, but repeatedly elevated levels of mean corpuscular volume (MCV) of red blood cells without a significant fall in hemoglobin levels—a rather unusual finding.

The present investigation therefore aimed to evaluate whether capecitabine therapy might be accompanied by an increase in MCV and, once we established this fact, to elucidate the possible pathomechanisms.

Patients and methods

Patients

A total of 154 patients were included from May 1998 up to December 2001. Out of these 154 patients, 115 were evaluated retrospectively and 39 prospectively.

Patients' demographics are shown in Table 1. All patients received a capecitabine-containing treatment regimen, either as monotherapy or in combination with other antineoplastic agents and/or immunotherapy. Capecitabine was administered in an outpatient setting and was given orally at a dose of 2500 mg/m^2 body surface area (BSA) daily divided into two doses for 14 days (i.e. one cycle), followed by 7 days of rest. This schedule was repeated every 3 weeks.

Table 1 **Patient characteristics**

Patients (M/F)	154 (72/82)
Age (±SD)	63 (±10)
Metastasized renal cell cancer	50
Metastasized breast cancer	41
Metastasized colorectal cancer	39
Metastasized pancreatic cancer	16
Others	8
Monotherapy	97
Concomitant chemotherapy	45
Concomitant immunotherapy	12
First-line therapy	50
Pretreated	104

Out of the 50 patients suffering from metastatic renal cell carcinoma, 38 patients with progressive disease after previous treatment with biological response modifiers ± cytotoxic chemotherapy received capecitabine monotherapy. The other 12 patients received capecitabine in combination with either s.c. interferon (IFN)-γ1b together with s.c. recombinant interleukin-2 as published previously [16] or IFN-α (6 MU/day) administered 3 times a week, as first-line palliative treatment.

All 41 patients with metastasized breast cancer were treated with capecitabine monotherapy as palliative third- or fourth-line therapy after having received standard anthracycline- and/or taxane-containing first- and second-line treatment.

Out of the 39 patients suffering from metastatic colon carcinoma, four patients received capecitabine as a single agent, the other 35 patients in combination with oxaliplatin 85 mg/m^2 (day 1) as first- or second-line palliative treatment regimen.

Sixteen patients suffering from metastatic pancreatic cancer received capecitabine in combination with gemcitabine 2200 mg/m^2 as palliative first-line treatment, whereas the remaining patients (four with unresectable hepatoma, one with advanced ovarian cancer, two with Klatskin's tumors and one 1 with gastric cancer) received capecitabine monotherapy as first-line palliative treatment.

Prior to each cycle of capecitabine therapy (i.e. every 3 weeks), in all patients a complete blood cell count including the red cell indices MCV, mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was performed on a Coulter STKS (Coulter Electronics, Krefeld, Germany). In the 39 patients investigated prospectively, additionally vitamin B_{12} and folic acid serum levels were determined by immunoassay on an Elecsys 2010 analyzer (Roche Diagnostics, Basel, Switzerland); total homocysteine in plasma was measured by a fluorescence polarization immunoassay on a IMX analyzer (Abbott, Abbott Park, IL)

Parameters were obtained during 3 consecutive cycles of therapy. After 9 weeks of capecitabine treatment, restaging was performed by use of either X-ray, ultrasound or computed tomography.

Statistical analysis

Results are given as median and interquartile range (IQR) from the 25th to the 75th percentile. Changes in

the investigated parameters during the entire observation period were calculated with the non-parametrical Friedman test and between two different points of time (or groups) with the Mann–Whitney test, respectively.

Results

The results reflect overall data obtained from all patients, since subgroup analyses regarding tumor entities or various antineoplastic regimen revealed no statistically significant differences.

Prior to the first cycle of capecitabine treatment, there were no abnormalities in red blood cells, white blood cells and platelets, respectively. Median hemoglobin levels (normal range 12–16 g/dl) were 12.3 g/dl (IQR 11.3–13.5), MCV (normal range 78–98 fl) was 84 fl (IQR 80.9–88.3), MCH (normal range 27–33 pg) was 28.9 pg (IQR 27.5–30.2) and MCHC (normal range 32–36 g/dl) was 33.8 g/dl (IQR 33.1–34.9).

Within 9 weeks, a statistically significant increase of MCV ($p < 0.0001$) could be observed up to 92.2 (IQR 89.4–97.2; Fig. 1). Neither MCH nor MCHC changed significantly. Moreover, hemoglobin levels did not change significantly either. Severe leukocytopenia or thrombocytopenia did not occur in any of the patients.

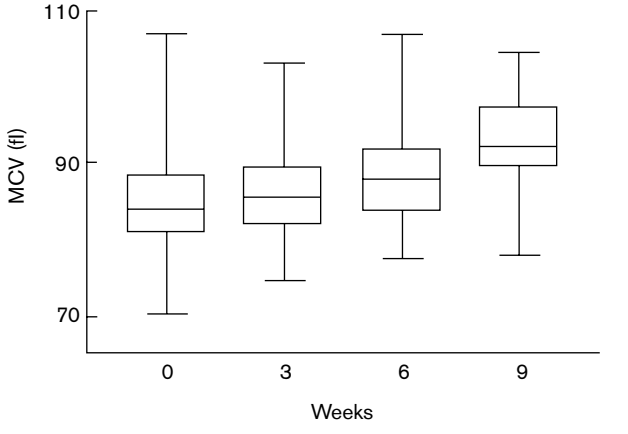
Once we established the fact of increasing MCV, we prospectively determined the vitamin B_{12}, folic acid and homocysteine levels in 39 patients. No significant alterations in these parameters could be observed during therapy (Table 2).

When comparing the rise of MCV (ΔMCV) over 9 weeks with response to capecitabine therapy (Fig. 2), ΔMCV tended to higher values in patients with complete or partial response ($n = 19$) or stable disease ($n = 112$) than in patients with tumor progression ($n = 23$). However, statistical significance could only be achieved between tumor response and tumor progression ($p < 0.03$; Table 3).

Discussion

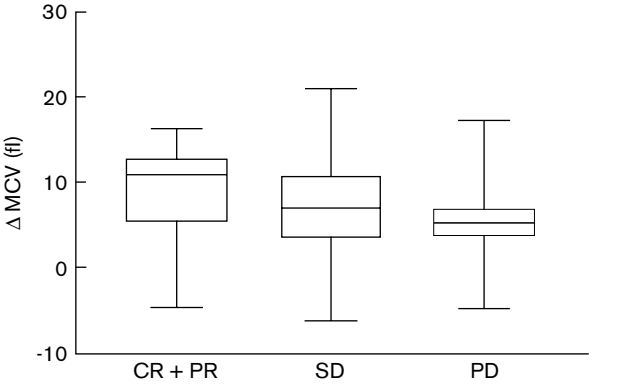
Capecitabine, a novel fluorouracil carbamate, is converted to 5-FU by a sequential triple enzyme pathway, located in the liver and tumor tissue. TP metabolizes 5-DFUR to 5-FU in the last step in the pathway and is therefore the most important enzyme for developing the antitumor activity of capecitabine. Tumor types known to have a high level of TP activity, such as renal cancer, are especially attractive targets for capecitabine therapy [15,17]. However, the cytotoxic activity of the metabolized 5-FU is mostly dependent on TS inhibition, which leads to a defective DNA synthesis.

Fig. 1



Time course of MCV levels during 9 weeks of capecitabine treatment (2500 mg/m^2/day). Data are presented as median and IQR from the 25th to 75th percentile.

Fig. 2



Increase in MCV over 9 weeks of capecitabine treatment (ΔMCV) with respect to tumor response (CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease). Data are presented as median and IQR from the 25th to 75th percentile.

Table 2 **Alterations in vitamin B_{12}, folic acid and homocysteine levels before and after 9 weeks of treatment with capecitabine**

Parameter	Normal range	Day 0 (IQR)[a]	Day 64 (IQR)[b]	*P*
Vitamin B_{12} (pmol/l)	118–720	313 (198–400)	293 (181–365)	NS
Folic acid (nmol/l)	3.4–38	17.0 (13.2–23.5)	19.3 (12.3–23.2)	NS
Homocysteine (μmol/l)	3–15	12.2 (11.1–12.0)	11.5 (9.3–14.2)	NS

Data are presented as median and IQR from the 25th to 75th percentile.
[a]Before capecitabine treatment.
[b]After 3 cycles of capecitabine.

Table 3 Comparison of MCV increase (ΔMCV) with respect to chemotherapy response

	CR + PR	SD	PD
ΔMCV	11.0 (IQR 5.2–12.7)	7.0 (IQR 3.3–10.0)	5.2 (IQR 3.5–6.6)
CR + PR	–	NS	$p < 0.03$
SD	NS	–	NS
PD	$p < 0.03$	NS	–

Data are presented as median and IQR from the 25th to 75th percentile. CR + PR = complete and partial response; SD = stable disease; PD = pro-progressive disease.

Hematologic abnormalities following antineoplastic therapy (anemia, leukopenia or low platelet counts) are a frequent finding in cancer patients; however, they also can occur in the absence of cytostatic treatment, especially anemia [18]. The differential diagnosis of anemias mainly includes hypoproliferative anemias, iron deficiency or anemia secondary to cancer therapy. These anemias are usually either normocytic and normochromic (MCV within normal range) or microcytic and hypochromic (decreased MCV). Rarely, megaloblastic (elevated MCV) anemias might also occur [19].

Megaloblastic anemia is the earliest sign of folic acid or vitamin B_{12} deficiency and is therefore seen in those patients who have impaired dietary intake of folate, tumor involvement or surgery of the small bowel or stomach. Folic acid or vitamin B_{12} deficiency results in defective DNA synthesis by the following pathomechanisms. Folate coenzymes are required for several biochemical reactions involved in synthesis of both purines and pyrimidines. Vitamin B_{12} (or homocysteine as an essential co-factor for the generation of vitamin B_{12}) deficiency appears to cause megaloblastic anemia as a result of an impaired folate metabolism which reduces thymidylate synthesis. The formation of cell DNA from thymidylate is therefore slowed down. This is responsible for the increased size of the cells in megaloblastic anemia, since the prolonged cell cycle would allow excess synthesis of RNA and other cytoplasmic components including hemoglobin [20].

Serum levels within the normal range rule out severe deficiencies of vitamin B_{12}, folic acid or homocysteine as an account of macrocytemia in our patients However, as mentioned above, all three of them are necessary for the generation of thymidylate, which, in last consequence, is mediated by TS, the same enzyme responsible for the cytotoxic action of 5-FU. Taking these facts in context, its seems plausible that the increase of MCV in patients treated with capecitabine might be due to the inhibition of TS also in erythroid precursor cells.

However, one could argue why this increase in MCV levels cannot be observed in patients treated with conventional (i.v.) 5-FU. We hypothesize the duration of application may be responsible. Following bolus 5-FU administration (as applied, for example, within the Mayo Clinic regimen on 5 consecutive days every 4 weeks), FdUMP could only be demonstrated up to 80 min in metastases of colon carcinomas [21], which could be verified by nuclear magnetic resonance spectroscopy [22]. In contrast, capecitabine, continuously administrated, results in continuous elevated intratumoral 5-FU levels [23], thereby most likely exerting permanent inhibition of TS.

Recent investigations found that TS polymorphism in peripheral blood cells may be used as a surrogate for intratumoral TS [24]. Taking this fact in context with our observation, i.e. patients responding to capecitabine treatment tending to higher MCV levels than patients not responding, one could hypothesize that TS inhibition in erythroid precursor cells corresponds with potent TS inhibition in tumor cells. However, it has to be conceded that statistical significance could be only be observed between tumor response and tumor progression, which might be due to different tumor entities and various treatment schedules, resulting in only small homogeneous subgroups. Therefore, further studies are warranted.

Summarizing our results, we conclude that an increased MCV without anemia in patients receiving capecitabine might be due to 5-FU-mediated inhibition of thymidylate synthesis, thus leading to slow formation of erythroid cell DNA. This effect seems to be independent of folic acid or vitamin B_{12} deficiency and does not require any therapeutic interventions. Whether an increase in MCV (or better ΔMCV in % of baseline values) might serve as surrogate marker for tumor response has to be determined in further investigations.

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