Changes in Folate Status as Determined by Reduction in Total Plasma Homocysteine Levels during Leucovorin Modulation of 5-Fluorouracil Therapy in Cancer Patients[1]

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ABSTRACT

We measured plasma total homocysteine (tHcy) in 14 patients (13 patients with colorectal cancer and 1 patient with breast cancer) during their first treatment with 5-fluorouracil (5-FU) plus leucovorin [LV (5-FULV)]. Eight of these patients were investigated a second time after 3–10 cycles (median, 4 cycles) with 5-FULV. Each cycle consisted of two administrations of 5-FU (500 mg/m^2) and LV (60 mg/m^2) given 24 h apart. The first administration of 5-FULV on day 1 of the first cycle induced a rapid reduction of the tHcy level from 12.5 μmol/liter (10.4–15.1 μmol/liter; geometric mean with 95% confidence interval of the mean) to 9.1 μmol/liter (7.5–11.1 μmol/liter) in 24 h. tHcy remained stable at this level after the second administration of 5-FULV. In addition, the 5-FULV regimen caused a concurrent 4-fold increase in both serum and erythrocyte folate. The fifth cycle with 5-FULV had only marginal effects on the tHcy level. 5-FU without LV modulation had no effect on the plasma tHcy or folate status in eight breast cancer patients. Our data establish the reduction of tHcy as a responsive indicator of LV pharmacodynamics.

INTRODUCTION

The combination of 5-FU[3] and LV was introduced in 1982 by Machover et al. (1, 2) for the treatment of metastatic colorectal cancer. Whereas monotherapy with 5-FU causes about a 10% response, the combined treatment (5-FULV) showed up to a 20–30% response in patients suffering from metastatic colo-

rectal cancers (3, 4). The mechanism behind this potentiation of the effect of 5-FU on the tumor cells is thought to be the stabilization of the complex between the target enzyme thymidylate synthetase and 5-fluoro-dUMP in the presence of high cellular levels of folate (5, 6).

Procedures for the assessment of the change in folate status induced by LV modulation have not been established. Such monitoring cannot be accomplished by the measurement of serum folate, which reflects recent folate intake rather than tissues stores (7, 8). Red cell folate is not adequate, because this parameter probably reflects the bone marrow folate status at the time of erythrogenesis (7, 8). Besides, both of these assays, particularly that for red cell folate, have low analytical precision (9).

The concentration of tHcy is a marker of tissue folate status. This is explained by the function of 5-methyltetrahydrofolate as a methyl donor in the reaction responsible for homocysteine remethylation to methionine (10). tHcy is elevated in folate-deficient subjects and decreased or normalized after 1–6 weeks of supplementation with folic acid at doses of 0.65–10 mg (11). Reduction of tHcy is also obtained in subjects with tHcy levels considered normal (5–15 μmol/liter), and the tHcy level in healthy adults in positive folate balance seems to approach levels of about 7–8 μmol/liter (12, 13). The usefulness of tHcy monitoring in clinical oncology is indicated by our previous observations that tHcy is a responsive indicator of the antifolate effect of methotrexate given at doses ranging from 25 mg to 13.6 g (14–17).

In the present study, we investigated the performance of tHcy as an indicator of the rapid alterations in folate status after the administration of LV at doses of 60 mg/m^2 as part of the 5-FULV regimen.

PATIENTS AND METHODS

Patients. Fourteen patients with either advanced colorectal cancer ($n = 13$; six females and seven males) or advanced breast cancer ($n = 1$) undergoing treatment with 5-FULV were enrolled in the study. The participants received their treatment at either the Department of Oncology of Haukeland University Hospital (Bergen, Norway; $n = 11$) or the Department of Oncology of the Norwegian Radium Hospital (Oslo, Norway; $n = 3$). Eight of these patients were investigated a second time after a median of 4 cycles (range, 3–10 cycles) with 5-FULV, corresponding to a median time of 8 weeks later. The baseline characteristics of these patients are given in Table 1. Eight patients with advanced breast cancer served as controls and were included to determine effects of 5-FU without LV modulation on tHcy.

All patients gave their written informed consent before participation, and the study protocol was approved by the local ethical committee.

Treatment. 5-FU was given as an i.v. continuous injection over a 5-min period at a dosage of 500 mg/m^2. Thirty min later, LV was administered at a dosage of 60 mg/m^2 as a bolus.

Table 1 Characteristics of patients and laboratory data at baseline

Patient no.	Age (yr)	Sex	Diagnosis[a]	Protocol[b]	Serum creatinine (μmol/liter)	Serum folate (nmol/liter)	Erythrocyte folate (nmol/liter)	Serum cobalamin (pmol/liter)	tHcy (μmol/liter)
1	48	F	CC	1	51	4.1	277	129	8.7
2	80	F	BC	1	137	8.0	345	134	21.4
3	64	M	CC	1, 2	107	10.5	573	182	14.2
4	72	M	CC	1, 2	80	5.9	507	355	9.8
5	60	F	RC	1, 2	95	22.1	588	2226	7.2
6	54	F	CC	1, 2	80	5.7	336	314	12.1
7	67	M	RC	1, 2	112	7.7	270	357	13.5
8	48	M	CC	1, 2	104	9.0	596	359	12.8
9	52	M	CC	1	133	12.3	1018	228	19.3
10	58	F	CC	1, 2	90	12.6	394	809	11.9
11	59	F	CC	1, 2	63	21.8	745	330	10.2
12	75	M	RC	1	81	NA[c]	NA	NA	19.2
13	83	M	CC	1	90	NA	NA	NA	15.3
14	49	F	CC	1	76	9.2	NA	970	8.9
Geo. mean[d]					90	9.5	473	367	12.5
95% CI					77–105	6.9–13.1	356–628	216–624	10.4–15.1

[a] CC, colon cancer; RC, rectal cancer; BC, breast cancer.

[b] 1, blood samples drawn before and during the first treatment with 5-FULV; 2, additional blood samples before and after the fifth (median) treatment with 5-FULV.

[c] NA, not available.

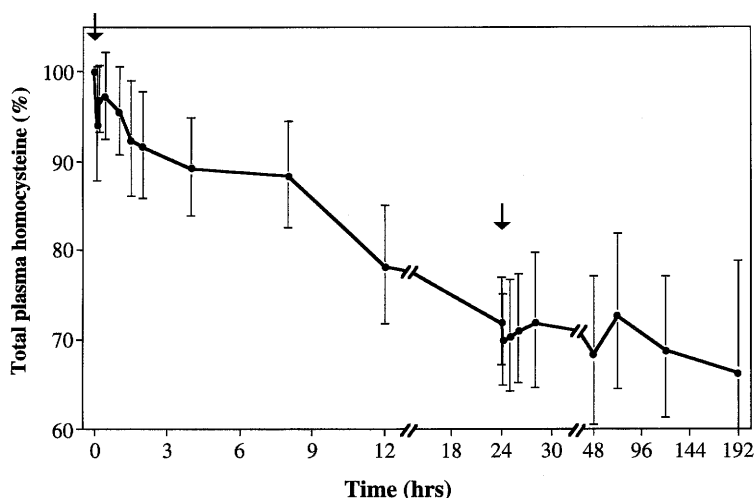[d] Geo. mean, geometric mean value.

Fig. 1 Reduction in tHcy during the first treatment with 5-FULV. Fourteen cancer patients were investigated, and the data are given as a percentage of the tHcy level measured immediately before treatment ($t = 0$). Results are given as the geometric mean values with 95% CI. *Arrows*, time of 5-FULV administration.

The treatment with 5-FULV was given on 2 consecutive days (at time 0 and 24 h; see Figs. 1 and 2) every 2 weeks.

The control patients received 5-FU at a dosage of 1000 mg/m^2 on days 1 and 2, as well as mitomycin (6 mg/m^2) on day 2.

Sampling Procedures. Blood was collected into Vacutainer tubes containing EDTA. Samples for tHcy measurements were placed on ice immediately after collection, blood cells were removed by centrifugation at 2500 rpm (10 min), and the plasma supernatant was frozen at −20°C until processing.

On the first day of the cycle, samples were drawn before treatment and after 5, 10, and 25 min as well as 1, 1.5, 2, 4, 8, and 12 h, starting immediately after the LV bolus. On the second day of treatment, samples were drawn before treatment and 10 min and 1, 2, 4, 8, 12, 24, 48, 72, 120, and 168 h after the LV bolus. All 14 patients were followed during the first treatment with 5-FULV, and 8 of these 14 patients were also investigated a second time, a median of 8 weeks later. Control patients were investigated on the first day of treatment while on 5-FU monotherapy.

Measurements. tHcy was measured using a fully automated method based on reduction with borohydride, derivatization with monobromobimane, and high-performance liquid chromatography separation. The between-day coefficient of variation of the assay is less than 5% (18). Serum and erythrocyte folate were assayed using the Quantaphase folate radioassay (Bio-Rad, Hercules, CA). The serum vitamin B$_{12}$ was determined with a microparticle enzyme intrinsic factor assay run on an IMX system (Abbott, Abbott Park, IL). Whereas tHcy was determined in every blood sample drawn, serum and erythrocyte folate as well as cobalamin and serum creatinine were determined only once before each treatment.

Statistics. Previous work from our group has shown that tHcy is log-normally distributed (13, 19). Accordingly, all values are given as the geometric mean values with 95% CIs. Values obtained before and during treatment were compared using the Wilcoxon matched pair signed rank test. All *P* values are two-tailed.

Fig. 2 tHcy levels during the fifth cycle (median range, 3–10 cycles) of treatment with 5-FULV. Eight patients with colorectal cancer were investigated, and the data are given as a percentage of the tHcy levels immediately before the first cycle. Results are given as geometric mean values with 95% CI. *Arrows*, time of 5-FULV administration.
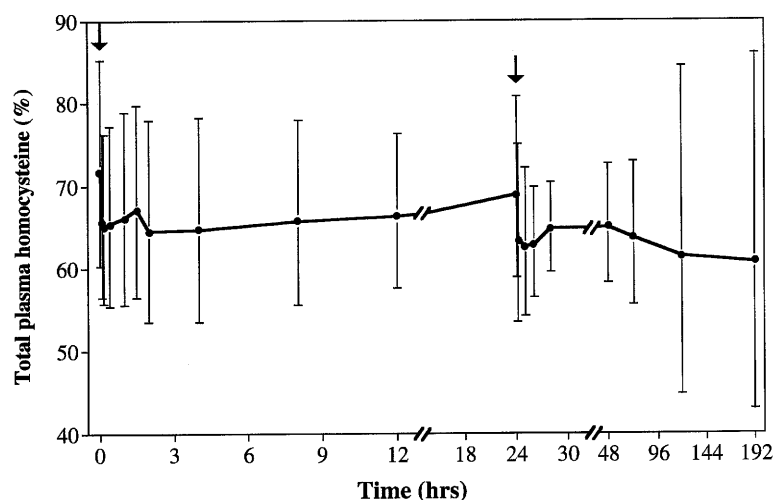
Table 2 Influence of 5-FULV treatment on the levels of tHcy cobalamin, serum folate, erythrocyte folate, and serum creatinine in patients with colorectal carcinomas ($n = 8$; geometric mean value with 95% CIs)

Parameter	Pretreatment	During 5-FULV treatment[a]	% of controls
tHcy (μmol/liter)	11.2 (9.3–13.5)	8.1 (6.9–9.4)[b]	71.7 (60.3–85.2)
Serum cobalamin (pmol/liter)	445 (235–845)	651 (420–1007)	146 (104–205)
Serum folate (nmol/liter)	10.5 (6.8–16.3)	42.4 (36.4–49.3)[c]	403 (282–577)
Erythrocyte folate (nmol/liter)	478 (359–635)	1455 (1240–1710)[c]	305 (238–391)
Serum creatinine (μmol/liter)	90.0 (76.7–106)	94.3 (84.8–104)	105 (95.3–115)

[a] After 4 (median) treatments with 5-FULV (range, 3–10 treatments).
[b] $P < 0.05$ *versus* pretreatment levels.
[c] $P < 0.01$ *versus* pretreatment levels.

RESULTS

Pretreatment Blood Indices. The patient group ($n = 14$) receiving 5-FULV had pretreatment levels of tHcy in the normal range (geometric mean level 12.5 μmol/liter; 95% CI, 10.4–15.1 μmol/liter). Serum folate, erythrocyte folate, cobalamin, and creatinine values are given in Table 1. One patient (patient 2) had low erythrocyte and serum folate levels combined with elevated tHcy (21.4 μmol/liter) and was probably folate deficient. All patients ($n = 8$) receiving 5-FU without LV modulation, who served as controls, had normal tHcy, creatinine, and vitamin levels (data not shown).

Effect of 5-FU with or without LV on Plasma tHcy. In patients receiving the first dose of the combination 5-FULV, tHcy declined rapidly at a rate of about 0.08 h⁻¹ (obtained by log-linear regression of the initial part of Fig. 1), reaching a plateau after 24 h at 9.1 μmol/liter (95% CI, 7.5–11.1 μmol/liter), which corresponds to about 70% of pretreatment levels (Fig. 1). The decrease of plasma tHcy reached a level of statistical significance ($P = 0.001$). The second dose of 5-FULV of the first cycle caused essentially no change in tHcy (Fig. 1).

Before the fifth cycle (median range, 3–10 cycles), the tHcy level was found to be at a low level of 8.1 μmol/liter (6.9–9.4 μmol/liter), and tHcy was not significantly changed after the first and second part of the next cycle (Fig. 2). The difference in the tHcy levels comparing the mean values measured immediately before the first and fifth (median) treatments with 5-FULV reached a level of statistical significance ($P < 0.05$).

In eight patients receiving 5-FU without LV, tHcy remained stable during treatment. The tHcy levels were 12.4 μmol/liter (95% CI, 9.2–16.7 μmol/liter) before 5-FU administration and 11.6 (95% CI, 8.6–15.8 μmol/liter) and 12.8 μmol/liter (95% CI, 9.6–17.0 μmol/liter) after 12 and 24 h, respectively.

Vitamins and Creatinine. Serum folate increased from a pretreatment value of 10.5 nmol/liter (CI, 6.8–16.3 nmol/liter; Table 2) before administration of the first 5-FULV cycle to 42.4 nmol/liter (95% CI, 36.4–49.3 nmol/liter) immediately before the fifth cycle. Erythrocyte folate increased from a pretreatment value of 478 nmol/liter (95% CI, 359–635 nmol/liter) to 1455 nmol/liter (95% CI, 1240–1710 nmol/liter). The increases in serum and erythrocyte folate levels were both statistically significant ($P < 0.01$). Serum creatinine and cobalamin levels were not significantly influenced by treatment with the 5-FULV regimen.

DISCUSSION

The enhanced antitumor effect of 5-FU achieved by LV modulation (3, 4) motivates the establishment of a laboratory marker to monitor the effect of LV on overall folate status. Such monitoring may be used to assess the tissue availability and metabolic effect of LV. More importantly, the effects of inter-individual difference on a folate marker such as tHcy should be compared with the therapeutic results in future clinical studies. Recent experimental studies (20) show that folate status at the time of initiation of 5-FULV treatment may influence the anti-tumor activity. However, the pretreatment tHcy level may not be

an accurate indicator of folate status in cancer patients, because the level may be influenced by tumor burden (16) and possibly by nutritional status and tissue catabolism.

We observed a rapid decline in plasma tHcy in patients receiving 5-FULV (Fig. 1). The effect on tHcy seems to be caused by LV only, because tHcy remained unchanged in patients treated with 5-FU monotherapy.

The tHcy reduction proceeded rapidly at an average rate of 0.08 h^{-1}, which corresponds to a half-life of about 9 h (Fig. 1). Furthermore, a stable plateau of about 8 μmol/liter was attained, and tHcy did not decrease further in response to additional LV administrations (Fig. 2).

The normal rate of tHcy removal from plasma is about 0.20 h^{-1} (21). The rate is not affected by folate status, and improved vitamin status lowers tHcy by decreasing homocysteine egress from tissues to plasma (22). Assuming that altered intracellular folate status immediately inhibits homocysteine egress, tHcy levels in plasma will change at a rate corresponding to plasma tHcy clearance. The fact that the rate of tHcy reduction induced by LV is much lower than the elimination rate from plasma suggests that folate distribution, uptake, or metabolism in tissues represents the rate-limiting step(s). Thus, the rapid tHcy reduction seems to be a responsive measure of increased tissue folate levels obtained by LV administration.

The stable tHcy plateau obtained after repeated LV doses (Fig. 2) equals the levels observed in healthy adults supplemented with folic acid for several weeks (11, 12) or those observed in subjects on a folate-rich diet over an extended period of time (13). Our finding emphasizes the importance of the folate dose, because maximal reduction in tHcy can be obtained within hours by the injection of 60 mg/m² LV (Fig. 1).

Summary and Conclusion. LV reduces tHcy at a rapid rate of 0.08 h^{-1}, and within 24 h, tHcy attains a stable, low level comparable to that observed in subjects with positive folate homeostasis. The kinetics of the tHcy reduction probably reflect the expansion of intracellular folate pool(s), which emphasizes its potential usefulness in monitoring LV pharmacodynamics. Furthermore, the rapid tHcy reduction also points to a novel application of LV in the management of clinical conditions in which immediate lowering of the tHcy level may be beneficial, such as, for example, in patients at high risk of a cardiovascular event (23).

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