were found for both sense and antisense single strand. It is likely that more than one stable conformation was formed for each single strand. In the case of the mutation, additional peaks were detected (Fig. 1).

To perform an automatic evaluation of the mutation, an important point is the reproducibility of the complex peak pattern. The analysis of different electrophoretic runs over 15 days confirmed the constant appearance of the peak pattern. Characteristic peaks for the wild-type and mutant alleles were defined by their retention time and height. For the sense strand (FAM), the peaks were defined by the following data points: wild-type 1, 5756; wild-type 2, 5809; mutant 1, 5736; mutant 2, 5788, and for the antisense strand (HEX): wild-type, 5867; mutant, 5738. In the antisense strand, one wild-type and one mutant peak were not considered for automatic evaluation because the two peaks partially overlapped. The variation of the peak retention time was less than ±8 data points for the sense strand and less than ±20 data points for the antisense strand.

The retention time of the peaks and their variations were listed in the Genotyper™ analysis software (Version 2.0; PE Applied Biosystems), and a macro was created for the analysis of the data (clear table; clear labels; select blue lanes; and select green lanes; label category peaks with the category's name; set up table with one category and one lane per row; append rows to table; show the table window; set cell row 1 column 1 to sample name; show the plot window). In the diagram, the peaks were named wild-type or mutant, and in the resulting table the summary of analysis was presented (Fig. 1). In the table, the peaks were named wild-type for the sense and antisense single strands in the case of the homozygous presence of the wild-type allele. In the case of homozygosity of the mutation, only peaks defined as mutant were recognized; in the heterozygous state, a combination of both was present. In routine usage, the practicability and reliability of the evaluation program has been demonstrated.

The ABI 310 Genetic Analyzer has been widely distributed in clinical laboratories in the last 2 years. The main applications of this system are sequencing and fragment analysis. In our report, we showed that SSCP analysis could also be performed with high reliability by capillary electrophoresis. For the first time, the combination of SSCP analysis and automatic evaluation of the mutation has been shown, even in cases where a complex SSCP peak pattern was present. In the diagnostic DNA testing, this approach is advantageous in the analysis of small sample numbers (as little as one sample).

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Kinetics of Plasma Total Homocysteine in Patients Receiving High-Dose Methotrexate Therapy, *Anne Berit Guttormsen,[1*] Per Magne Ueland,[1] Per Eystein Lønning,[2] Olav Mella,[2] and Helga Refsum[1]* ([1] Department of Pharmacology, University of Bergen, Armauer Hansens hus, N-5021 Bergen, Norway; [2] Department of Oncology, Haukeland University Hospital, N-5021 Bergen, Norway; * author for correspondence: fax 47 55 974605, e-mail Anne.Guttormsen@farm.uib.no)

Homocysteine (Hcy) is a sulfur amino acid formed from methionine during transmethylation. Once formed, it is either remethylated to methionine or irreversibly catabolized to cystathionine. The remethylation is catalyzed by methionine synthase (EC 2.1.1.13), which requires cobalamin as cofactor and 5-methyltetrahydrofolate as substrate *(1)*. This explains why the fasting total homocysteine (tHcy) concentration is related to overall folate or cobalamin status and that increased tHcy has been used to diagnose deficiencies of these vitamins *(2–4)*.

Methotrexate (MTX) is an antifolate drug that inhibits dihydrofolate reductase, thereby depleting the cells of reduced folates, including 5-methyltetrahydrofolate *(5)*. In cell culture studies, MTX inhibits Hcy remethylation, leading to increased Hcy export to the medium *(6–8)*.

Plasma tHcy is a sensitive indicator of the antifolate effect of MTX, as demonstrated by an increased plasma tHcy concentration. The increase is maximal after ~2 days in psoriasis patients given only 25 mg of MTX *(9)*. In cancer patients receiving intermediate or high doses of MTX, there is a rapid increase in plasma tHcy within hours. However, the increased tHcy induced by MTX is normalized after rescue therapy with folinic acid *(7, 10)*.

We have previously shown that tHcy clearance in folate-deficient subjects with hyperhomocysteinemia is close to that observed in healthy individuals *(11)*. However, it has been suggested that folate status predominately affects concentrations of fasting, i.e., low tHcy concentrations. Therefore, the present study was undertaken to examine tHcy elimination in subjects with plasma tHcy within reference values and to investigate whether the antifolate effect of MTX had any influence on plasma clearance. This was carried out by monitoring elimination of [^{14}C]Hcy injected intravenously in a dose that did not affect basal tHcy concentrations. By this procedure, elimination kinetics of tHcy at low, fasting tHcy concentrations are obtained.

The study group comprised six male cancer patients (mean age ± SD, 40 ± 19 years; Table 1) recruited from the Department of Oncology, Haukeland University Hospital, Bergen, Norway. The protocol was approved by the

Table 1. Patient characteristics, plasma indices,[a] and kinetic variables.

Patient	Age, years	Weight, kg	tHcy, μmol/L		Creatinine, μmol/L		s-cob,[b] pmol/L		s-folate, nmol/L		$T_{1/2}$, h		Cl, mL/min		V_d, L/kg	
			−	+	−	+	−	+	−	+	−	+	−	+	−	+
AA	45	67	6.5	11.8	85	94	871	443	27.6	>45.3	2.8	2.5	79	75	0.29	0.24
BB	23	95	7.8	5.3	104	87	590	607	>45.3	>45.3	2.1	2.8	92	98	0.27	0.25
CC	22	70	7.6	3.5	113	104	401	535	9.5	>45.3	2.6	2.7	73	76	0.23	0.25
DD	51	97	7.7	9.4	109	95	564	549	17.7	>45.3	3.5	3.7	84	75	0.26	0.25
EE	29	70	7.8	7.4	92	84	242	255	16.1	>45.3	2.1	2.4	76	60	0.32	0.28
FF	70	58	13.9	20.8	83	96	180	464	8.9	>45.3	2.7	3.6	62	63	0.24	0.39
Mean	40	76	8.6	9.7	98	93	475	476			2.6	3.0	78	75	0.27	0.28
SD	19	16	2.7	6.2	13	7	255	123			0.5	0.6	10	13	0.03	0.06

[a] The blood samples were collected immediately before tracer injection in both study situations.

[b] s-cob, serum cobalamin; s-folate, serum folate; −, without methotrexate; +, with methotrexate; Cl, clearance; and V_d, distribution volume.

Regional Ethics committee in health region III, and all patients gave their written informed consent at inclusion.

The patients received one of two cytostatic regimens, both including high-dose (HD)-MTX. Patients AA, DD, EE, and FF (non-Hodgkin lymphoma patients) had eight courses of CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) and six courses of HD-MTX given ~10 days after the CHOP regimen. Patients BB and CC (medulloblastoma patients) had peroral procarbazin (days 1–14), vincristine (days 1, 8, 15, 22, 29, and 36), and HD-MTX courses (days 15, 22, and 29). When included in the present study, the patients had received none or one course of HD-MTX.

The kinetics of [^{14}C]Hcy were investigated on two occasions in each patient, i.e., both without and during HD-MTX treatment (a 2-h infusion of MTX started ~12 h before tracer injection). L-[1-^{14}C]Hcy was prepared immediately before use from L-[1-^{14}C]Hcy thiolactone (56 mCi/mmol, Amersham International) as described *(12)*; a bolus of 200 μCi of [^{14}C]Hcy (3.6 μmol) in 20 mL of 250 mmol/L NaCl, pH 5, was injected within 1 min. Blood samples were collected for 24 h, and tHcy was determined *(13)*.

Plasma samples were reduced, derivatized, and quantified according to a slight modification of an automated assay for tHcy *(13)*. Plasma samples (360 μL) were mixed with 4 mol/L sodium borohydride (200 μL, in 0.1 mol/L NaOH), and then 5 mol/L HCl (40 μL) was added. After 10 min, protein was precipitated by adding 200 μL of 2 mol/L sulfosalicylic acid. The acid supernatant (240 μL) was mixed with of 6.7 mmol/L dithioerythritol (60 μL), 1 mol/L NaOH (150 μL), 4 mol/L sodium borohydride (150 μL), 5 mmol/L EDTA (60 μL), water (2100 μL), and 25 mmol/L monobromobimane (60 μL). The derivatization was stopped after 3 min by adding 210 μL of glacial acetic acid. The Hcy-bimane adduct was separated and quantitated using reversed-phase chromatography as previously described *(13)*. For each plasma sample, six consecutive injections (each 400 μL) were performed, and the eluate containing the labeled Hcy adduct was collected and pooled. The pooled eluate was evaporated to dryness and dissolved in water and scintillation fluid; the radioactivity was determined by scintillation counting (Packard Tri-Carb 300, United Technologies).

Serum cobalamin and serum folate were determined as reported elsewhere *(12)*.

The elimination of tHcy after intravenous injection obeys first order kinetics and is consistent with a two-compartment model *(14)*. However, for simplicity and comparison with our previous studies *(11, 12, 15)*, the elimination rate constant (k_e) and half-life ($T_{1/2}$) were calculated by linear regression of the terminal, linear part (2–6 h) of the log-transformed concentration vs time curve *(15)*. The kinetic variables were calculated using KaleidaGraph™, Ver. 2.1.3 for Macintosh (Synergy Software). The time course for tHcy was also analyzed by the program PCNONLIN, Ver. 4.0 (Statistical Consultants Inc.) based on the Akaike's information criterion *(16)* for the best curve fit. The $T_{1/2}$ obtained by these two methods differed by <20% in most patients. The formulas used *(17)* for the calculations are given elsewhere *(12)*.

The results are given as mean and SD. Comparison of paired data was performed using the Wilcoxon signed-rank test, and unpaired values were compared using the Mann–Whitney *U*-test.

The elimination kinetics of plasma tHcy were investigated in six cancer patients on two occasions, before and during HD-MTX treatment. Patient characteristics and blood indicators obtained immediately before each investigation are listed in Table 1. All patients had serum folate and cobalamin above the reference ranges and serum creatinine concentrations within reference values. tHcy concentrations were 8.6 ± 2.7 μmol/L and 9.7 ± 6.2 μmol/L before the first (−MTX) and second studies (+MTX), respectively (Table 1).

Within the first 15 min after the injection of the [^{14}C]Hcy tracer, there was essentially no change in tHcy (<1 μmol/L). Thus, this test condition does not influence the tHcy concentration and therefore allows the assessment of plasma tHcy elimination kinetics at low, fasting concentrations. In contrast, the standard dose of unlabeled Hcy used for the Hcy loading test causes a 60–100 μmol/L increase in plasma tHcy *(11, 12, 15, 18)*.

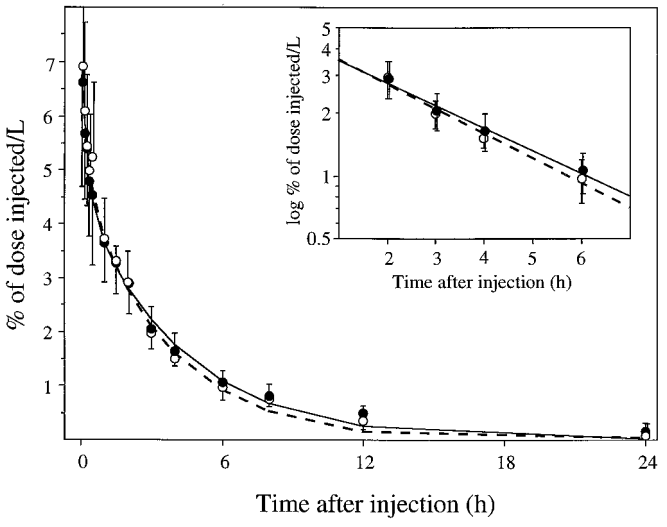

Fig. 1. Elimination kinetics of [^{14}C]Hcy from plasma in six cancer patients.

The kinetics were determined without concurrent MTX treatment (○) and during MTX treatment (●) by injecting [^{14}C]Hcy, as described. The results are given as mean with SD as *bars*. The computer program PCNONLIN was used for curve fitting. Curves without (– – –) and with (———) MTX treatment are shown. The *inset* shows the corresponding log-linear regression lines in the time interval 2–6 h.

Plasma tHcy remained essentially stable for 24 h in the absence of MTX. Only a minor increase of 12.7% ± 9.3% (1.1 ± 0.8 μmol/L) was observed 8 h after the injection, a diurnal change that is in the same range as previously reported in healthy individuals *(18)*. In contrast, there was a variable but substantial increase in tHcy in patients after MTX infusion, reaching a maximum of 49.3% ± 61.6% (3.0 ± 2.4 μmol/L) after 12 h, demonstrating the antifolate effect of MTX. Similar effects on plasma tHcy has been reported previously *(10, 19, 20)*.

In the absence of MTX, the plasma $T_{1/2}$ was 2.6 ± 0.5 h (k_e = 0.27 ± 0.03), corresponding to a clearance of 78 ± 10 mL/min. Plasma tHcy kinetics showed no consistent changes in response to HD-MTX. The mean differences in $T_{1/2}$ and clearance between the two occasions were 13.3% ± 17.5% and −4.1% ± 10.4%, respectively ($P > 0.05$; Fig. 1 and Table 1).

From these data, the following conclusions can be made: (*a*) the plasma tHcy kinetics are not affected by HD-MTX and thereby folate status, as previously demonstrated by Hcy loading in folate-deficient subjects *(11)*; and (*b*) the kinetics are similar albeit slightly more rapid than observed during Hcy loading ($T_{1/2}$= 2.6 vs 3.7 h; P = 0.008) *(12)*. These results verify that peroral Hcy loading is an adequate procedure for the assessment of Hcy turnover in plasma.

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Improved Method to Retrieve DNA from Dried Silver-stained Polyacrylamide Gels, *Steven P. Siembieda and David J. Lakatua** (Regions Hospital, St. Paul, MN 55101; * address correspondence to this author at: Regions Hospital, Department of Pathology, 640 Jackson St., St. Paul, MN 55101; fax (612) 221-2741, e-mail David.J.Lakatua@Healthpartners.com)

Differential display analysis (DDA) has become a useful and popular technique to identify differentially expressed