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Original Paper

Intermittent Continuous Infusion of 5-Fluorouracil and Low Dose Oral Leucovorin in Patients with Gastrointestinal Cancer: Relationship between Plasma Concentrations and Clinical Parameters

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Modulation of 5-fluorouracil (5-FU) by leucovorin and continuous infusion of 5-FU can both result in enhanced therapeutic efficacy. The main objective of this study was to determine the maximum tolerated dose (MTD) of oral leucovorin in combination with continuous infusion of 5-FU for 14 days every 4 weeks at a dose of 300 mg/m^2/day in 30 patients with gastrointestinal cancer. The MTD of oral leucovorin was established at 10 mg/day. Dose-limiting toxicities were mucositis, diarrhoea and hand–foot syndrome. Plasma leucovorin concentrations were below the detection limit of the assay (<0.5 μM). Plasma 5-FU concentrations varied considerably from 0.06 to 11.3 μM. A relation between toxicity, response and plasma concentration of 5-FU could not be established. Our data may indicate that even very low plasma concentrations of leucovorin are able to modulate 5-FU. In 17 patients with colorectal cancer the response rate was 24% (95% CI: 7–50%), which is comparable to other treatment schedules with leucovorin or to continuous infusion of 5-FU alone.

Key words: chemotherapy, colorectal cancer, continuous infusion, 5-fluorouracil, gastrointestinal cancer, leucovorin

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INTRODUCTION

IN PATIENTS with disseminated colorectal cancer, response rates of only 10–20% have been reported after bolus injection with 5-fluorouracil (5-FU) [1, 2]. Recently some progress has been made by biochemical modulation of 5-FU [2–5] or by expanding the infusion time of 5-FU [1, 6–10].

So far, biochemical modulation with intravenous leucovorin seems to have the best results [2, 11, 12], which is confirmed by a meta-analysis of all known randomised studies comparing 5-FU and leucovorin to 5-FU alone [13]. No clear dose–response effect has been reported for leucovorin and identical response rates are obtained when intravenous leucovorin is replaced by oral leucovorin [14–19], although the latter result has not been confirmed in a direct phase III comparison.

Prolonged infusion of 5-FU is another way to improve the efficacy of 5-FU as shown in several randomised studies comparing continuous infusion with bolus injection of 5-FU [1, 6–10].

In a previous study at our institution using an intermittent continuous infusion schedule of escalating doses of 5-FU, no dose–response relation could be established. The overall response rate in that study was 11% (95% CI: 1–33%) [20]. In order to improve the results of that study, we started the present study in patients with gastrointestinal cancer with intermittent continuous infusion of a fixed dose of 5-FU combined with escalating doses of oral leucovorin. The objectives were to determine the maximum tolerated dose of oral leucovorin and to examine if a relationship could be established between the plasma concentrations of 5-FU, leucovorin and response and toxicity. For the subgroup of patients with colorectal cancer the response rate was determined.

PATIENTS AND METHODS

Patients

30 patients with metastatic gastrointestinal cancer entered the study. The patient characteristics are shown in Table 1; 20

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patients had colorectal cancer, 10 patients had other forms of gastrointestinal cancer. All patients had progressive measurable disease. Measurable disease was defined as a lesion measurable in two dimensions by computed tomography, ultrasound, chest X-ray or physical examination. Previous chemotherapy was not allowed. Radiotherapy was allowed, provided that the indicator lesion was outside the radiation field. Patients had to have a WHO performance status of ≤ 2, a leucocyte count of $\geq 4.0 \times 10^9$/l and a platelet count of $\geq 100 \times 10^9$/l. Patients were ineligible for this study if they had any other malignancy, except basal cell carcinoma of the skin. Pretreatment assessment included a medical history, physical examination, tumour measurements, chest X-ray, electrocardiogram, complete blood cell count and blood chemistries.

Treatment

In all patients a subcutaneous infusion chamber (Port-a-Cath®) was implanted. Patients were treated with continuous infusion of 5-FU for 14 days every 4 weeks at a dose of 300 mg/m^2/day via a portable infusion pump (Parker® micropump or Pharmacia CADD-PLUS® pump). During the 5-FU infusion, oral leucovorin (Lederle) was administered at a dose of 5 mg/day in the first 6 patients and was then escalated by 5 mg/day in every subsequent group of 6 patients until toxicity. The maximum tolerated dose (MTD) of leucovorin was defined as the dose of leucovorin that caused any toxicity > grade 2 (WHO) (except for mucositis and hand–foot syndrome for which grade 1 applied) in 3 out of 6 patients.

In the case of toxicity > grade 2, for mucositis and hand–foot syndrome > grade 1, or otherwise unacceptable toxicity for the patient, initially the dose of 5-FU was reduced by 25% and the dose of leucovorin left unchanged. If toxicity still persisted, the dose of leucovorin was subsequently reduced by 5 mg/day. Treatment was stopped in the case of disease progression, patient refusal or unacceptable toxicity to the patient.

Response and toxicity evaluation

Patients were considered evaluable for response after at least two courses. Objective response was defined according to standard WHO criteria. Toxicity evaluation took place every 2 weeks. Toxicity was scored according to WHO toxicity grades [21].

Measurement of 5-FU and leucovorin in plasma

Plasma samples to measure steady state concentrations of 5-FU and leucovorin were taken on days 7 and 14 in the first 12 patients and on day 14 in all subsequent patients.

5-FU for analytical procedures was obtained from Sigma (St Louis, Missouri, U.S.A.); pentafluorobenzyl-bromide from Pierce Chemicals (Rockford, Illinois, U.S.A.). ^{15}N,[^{15}N]fluorouracil (purity 99.9%) was obtained from Merck-Sharp and Dohme (Montreal, Canada). All other chemicals were of analytical grade. Solutions were made in water purified by a Millipore Reagent Q system (Millipore, Bedford, Massachussetts, U.S.A.). All plasma concentrations of 5-FU were measured with gas chromatography coupled to mass spectrometry (GC–MS), essentially as described previously for human plasma samples [22]. However, for the present samples we exchanged the internal standard 5-chlorouracil for ^{15}N,[^{15}N]5-FU and samples were further processed as described previously [23].

Details of the GC–MS conditions have been previously reported [22, 24]. The resulting detection limit for tissue samples was 1 pmol/ml. By using a resolution of the mass spectrometer of 3000 (10% valley), the absolute sensitivity of the method was 1 pg injected to the GC–MS system. The accuracy and sensitivity was improved by suppressing the chemical noise. The concentrations of leucovorin and 5-methyltetrahydrofolate were measured as described previously by Trave and associates [25].

Statistical analysis

To determine the difference in plasma concentrations of 5-FU between days 7 and 14, a mixed model analysis of variance was used with the difference between days 7 and 14 as a fixed effect, the course number as a covariate and with patients as random factor. To determine the relationship between plasma concentrations of 5-FU and toxicity, analysis by the random effects logistic-binomial regression model for distinguishable data was performed (Egret Statistical Software Package, Statistic and Epidemiology Research Corporation).

RESULTS

30 patients entered the study. A total of 144 courses (median 4, range 1–16) were given with 1864 infusion days representing 92% of the planned infusion duration. 3 patients were ineligible for this study because of pretreatment with continuous infusion of 5-FU. These patients were however evaluable for toxicity and for measurement of plasma levels of 5-FU and leucovorin. All 30 patients were evaluable for toxicity. The first 6 patients received 5 mg of oral leucovorin daily for 14 days, and the next 6 patients received 10 mg/day (Table 2).

By definition, according to these results, the maximum tolerated dose of oral leucovorin in this schedule was established at 10 mg/day. All other 18 patients thus received 10 mg of oral leucovorin each day. Because of toxicity, dose reduction of 25% of the 5-FU was performed in 3 of the 6 patients who received 5 mg of oral leucovorin. Dose reduction of 25% of the 5-FU was necessary in 13 of the 24 patients who received 10 mg of oral leucovorin. In 5 of the 13 patients toxicity decreased after 25% 5-FU dose reduction. In the other 8 patients toxicity persisted. Eventually 5 of these patients had a 5 mg dose reduction of the oral leucovorin. 2 of these 5 patients had persistent toxicity and

Table 1. Patient characteristics

Characteristics	No
Sex	
Male	12
Female	18
Median age in years (range)	56 (29–72)
Primary tumour	
Colon/rectum	20
Pancreas	7
Stomach	1
Small intestine	1
Unknown	1
Site of indicator lesion	
Liver	22
Lung	2
(Retro)peritoneum	2
Primary tumour	4
Performance status (WHO)	
0	11
1	15
2	4

Table 2. Toxicity

	Leucovorin dose					
	5 mg (n = 6)			10 mg (n = 6)		
Grade (WHO)	1	2	3	1	2	3
Mucositis	3	1	0	2	1	0
Diarrhoea	2	1	0	3	2	0
Nausea	1	0	0	1	1	1
Hand–foot syndrome	0	0	1	0	1	0

Overall toxicity in the first two groups of 6 patients with escalating dose of oral leucovorin; n, number of patients.

Table 3. Toxicity

	Leucovorin dose					
	5 mg (n = 36)			10 mg (n = 104)		
Grade (WHO)	1	2	3	1	2	3
Mucositis	17	2	0	15	9	0
Diarrhoea	11	6	0	9	5	1
Nausea	6	3	3	4	7	1
Hand–foot syndrome	3	0	0	13	5	1
Skin	3	0	0	3	0	0
Leucopenia	0	0	0	6	3	0

Toxicity in percentage of number of courses; n, number of courses.

leucovorin was, therefore, stopped in these 2 patients (4 courses). One patient developed an acute myocardial infarction on the day of the start of the sixth course of 5-FU infusion in combination with 5 mg/day of oral leucovorin. Overall, some form of toxicity occurred in 87% of the patients; this was in 52% of the courses administered (Table 3).

27 patients were evaluable for response (Table 4). Of the patients with colorectal cancer, one had a complete response after 8 months with a duration of 2 months; 3 patients had a partial response after 2, 4 and 6 months with a duration of 13, 8 and 6 months, respectively. 10 patients had stable disease for a median duration of 4 months (range 3–14 months). 3 patients progressed after one or two courses.

The median survival of patients with colorectal cancer was 10 months (range 1–22 months), for responders 17 months (range 13–22 months). The median survival for the whole group was 9 months (range 1–22 months).

Plasma samples taken at days 7 and 14 were evaluable for 12 patients. All samples were taken at the same time of day to exclude a possible circadian variation in plasma 5-FU concentrations as has been described previously in patients by Harris and associates [26] and in mice [27]. Figure 1 shows a typical example of the 5-FU concentration measured in one patient during four courses; a clear increase in the 5-FU concentration at the end of the infusion was observed. Since the study was performed on the basis of an outpatient protocol, in subsequent patients only sampling at day 14 was scheduled, but only samples from 10 of the 18 patients were evaluable. For several patients it was investigated whether it was possible to use samples taken from the Port-a-Cath® infusion system; however, despite extensive flushing with saline, 5-FU was retained in the tubes preventing any reliable measurement in samples taken in this way; all values were significantly higher (up to >100 μM) than samples simultaneously taken from the peripheral vein. Plasma 5-FU concentration in all patients at day 7 varied from 0.06 to 10.22 μM, and at day 14 from 0.18 to 11.3 μM. Pooling all data

Table 4. Response rate

Response rate	Gastrointestinal cancer (n = 27)	Colorectal cancer (n = 17)
Complete response (CR)	1	1
Partial response (PR)	3	3
Stable disease	16	10
Progressive disease	7	3
Overall response (CR + PR)	15% (95% CI: 4–34%)	24% (95% CI: 7–50%)

n, number of patients.

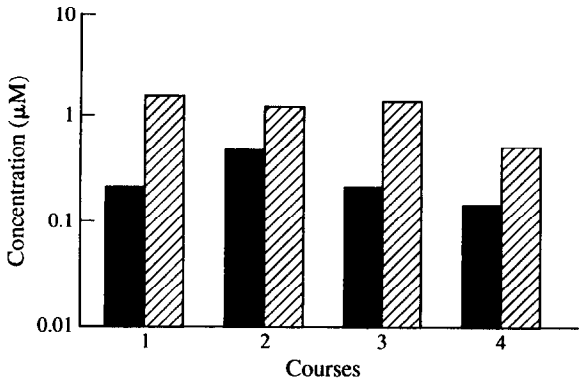

Figure 1. Plasma 5-FU concentrations in one patient during four subsequent courses of 5-FU. Samples were taken at day 7 (solid bar) and at day 14 (hatched bar) at the same time of the day.

from the patients clearly demonstrated that the mean plasma concentrations of 5-FU were significantly higher ($P = 0.001$) at day 14 (2.86 μM) than at day 7 (0.70 μM). Since all leucovorin concentrations were below the detection limit of the assay (<0.5 μM) we did not attempt to discriminate between the D- and L forms of leucovorin. Statistical analysis showed no relation between plasma 5-FU concentrations and toxicity; also no relation with response rate could be established.

DISCUSSION

Intermittent continuous infusion of 5-FU in combination with oral leucovorin is feasible on an outpatient basis. In the study we used oral leucovorin for several reasons. Pharmacokinetic studies showed a potential advantage for oral compared with intravenous administration [28]. Furthermore similar response rates have been reported in phase II studies when intravenous infusion of leucovorin is replaced by high dose oral leucovorin [14–17]. Another reason was the ease of incorporation of oral leucovorin into an outpatient-based schedule of intermittent continuous infusion of 5-FU.

The MTD of oral leucovorin in our study was established at 10 mg/day. Two other studies also combined continuous infusion of 5-FU with oral leucovorin [18, 19] (Table 5). The MTD of oral leucovorin in one study was established at 20 mg every 8 h [18]. The other study used a fixed dose of oral leucovorin [19]. The dose-limiting toxicities in these studies were also mucositis, diarrhoea and hand–foot syndrome, a pattern comparable to continuous infusion schedules of 5-FU alone [6, 7, 29, 30]. However, the incidence of toxicity is higher and toxicity is also more severe. It can, therefore, be concluded from these studies that the maximum tolerated dose of oral leucovorin is strongly schedule-dependent and is mainly determined by the dose and duration of 5-FU infusion. One patient in our study developed an acute myocardial infarction occurring at the start of the sixth course of 5-FU infusion. This patient had no history of ischaemic heart disease. Cardiotoxicity of 5-FU in the form of ischaemia has been reported [31]. The incidence is low and somewhat higher in patients with previously documented ischaemic heart disease [32].

In our study all responses were seen in the subgroup of patients with colorectal cancer. The response rate of 24% is comparable to studies with a bolus injection of 5-FU in combination with an intravenous infusion of leucovorin [2, 13] and also to continuous infusion of 5-FU alone [1, 6–10, 30]. In two other studies the combination of continuous infusion of 5-FU with oral leucovorin differed especially in the duration of the 5-FU infusion. This may be the reason for the difference in response rate, when compared with our study [18, 19] (Table 5).

All responses in our study occurred in the group of patients who received 10 mg of leucovorin. However, in 2 of the 4 patients a dose reduction of 5-FU and leucovorin was necessary before the response was achieved. No dose–response relationship of oral leucovorin could be established, because of the low number of responders. Other studies using different schedules of 5-FU and leucovorin also did not find a clear dose–response relationship [2, 14–19].

The explanation for the absence of a relation between the dose of leucovorin and response could be due to the fact that inhibition of thymidylate synthase occurs if intracellular folates exceed a certain threshold level, and that when this threshold level is reached, further dose escalation does not lead to a higher response rate [33].

It is not precisely known what concentration of leucovorin is necessary for maximal 5-FU binding and inhibition of thymidylate synthase, resulting in an increase of cytotoxicity [33, 34]. *In vitro* studies report variations from 0.1 to 10 μM leucovorin [35, 36].

Although plasma concentrations of leucovorin were below the detection limit of the assay, this may be enough to result in a modulatory effect, because we observed an increased toxicity of this schedule compared with other schedules with 5-FU alone; however, no relation to antitumour activity could be established. A similar lack of correlation has been reported in two other studies with continuous infusion of 5-FU and oral leucovorin [18, 19]. In two other studies, the level of leucovorin concentrations measured was strongly dependent on the dose of leucovorin [14, 17]. 5-FU concentrations in our study were measured

Table 5. Oral leucovorin and continuous infusion of 5-fluorouracil (5-FU)

5-FU dose	Leucovorin dose per day	Number of patients	Response rate (%)	Ref.
100 mg/m²/day (protracted infusion) (fixed dose)	15 mg in 3 doses (escalation until toxicity) (max. 67.5 mg)	27	–	[18]
225 mg/m²/day (protracted infusion) (escalating)	5 mg/m² (fixed dose)	21	71* (9 CR, 62 PR)	[19]

max., maximum; *95% CI: 48–89%; CR, complete response; PR, partial response.

on days 7 and 14. There was a significant difference in the plasma concentrations between days 7 and 14, although a large variation was observed. A possible explanation for this large variation in plasma 5-FU concentrations is described by Etienne and associates [37]. By using a portable pump with cartridges containing concentrated 5-FU solutions, plasma levels may vary tremendously, owing to the concentration of the 5-FU solution and the prolonged interval (compared with non-portable pumps) between the pulses of the portable pump. Diasio and Harris reported that no clear relation could be established between the plasma concentration and 5-FU doses, although different doses (varying from 300 mg/m^2/day to 175 mg/kg/day) were used. Plasma concentrations varied from 0.8 to 71 μM [38].

Statistical analysis of our own data did not reveal a relation between plasma 5-FU concentrations and toxicity. Hillcoat and colleagues found, however, an indication of a positive correlation between plasma 5-FU concentrations and tumour response, although plasma concentrations of 5-FU varied widely in that study [39]. However, recently Presant and associates postulated a relationship between tumour half-life for 5-FU and response of patients treated with a 5-FU regimen [40], while we observed a relationship between plasma 5-FU AUC and toxicity [22].

It can be concluded from our study that the addition of oral leucovorin to an outpatient-based schedule of continuous infusion of 5-FU is feasible. The MTD of oral leucovorin in this schedule was established at 10 mg/day. A relationship between toxicity, response rate and plasma concentrations of 5-FU could not be made, but it seems likely from the increased toxicity that even very low plasma concentrations of leucovorin are able to modulate 5-FU. The response rate of 24% is comparable to studies with continuous infusion of 5-FU alone [1, 6–10] or with schedules using bolus injection of 5-FU and short-term intravenous infusion of leucovorin [2, 11–13, 30].

1. Hansen RM. Systemic therapy in metastatic colorectal cancer. *Arch Intern Med* 1990, **150**, 2265–2269.
2. Peters GJ, Van Groeningen CJ. Clinical relevance of biochemical modulation of 5-fluorouracil. *Ann Oncol* 1991, **2**, 469–480.
3. Wadler S, Schwartz EL, Goldman M, *et al.* Fluorouracil and recombinant alfa-2a-interferon: an active regimen against advanced colorectal carcinoma. *J Clin Oncol* 1989, **7**, 1769–1775.
4. O'Dwyer PJ, Paul AR, Walczak J, *et al.* Phase II study of biochemical modulation of fluorouracil by low dose PALA in patients with colorectal cancer. *J Clin Oncol* 1990, **8**, 1497–1503.
5. Kemeny N, Israel K, Niedzwiecki D, *et al.* Randomized study of continuous infusion fluorouracil versus fluorouracil plus cisplatinum in patients with metastatic colorectal cancer. *J Clin Oncol* 1990, **8**, 313–318.
6. Seifert P, Baker LH, Reed ML, Vaitkevicius VK. Comparison of continuously infused 5-fluorouracil with bolus injection in treatment of patients with colorectal adenocarcinoma. *Cancer* 1975, **36**, 123–128.
7. Lokich JJ, Ahlgren JD, Gullo JJ, Philips JA, Fryer JG. A prospective randomized comparison of continuous infusion fluorouracil with a conventional bolus schedule in metastatic colorectal carcinoma: a Mid-Atlantic Oncology Program Study. *J Clin Oncol* 1989, **7**, 425–432.
8. Hansen R, Ryan L, Anderson T, Quebbeman E, Haller D. A phase III trial of bolus 5FU versus protracted infusion 5FU +/− cisplatin in metastatic colorectal cancer. An Eastern Cooperative Oncology Group Study (EST 2286). *Proc Am Soc Clin Oncol* 1992, **11**, 171 (abstract).
9. Weinerman B, Shah A, Fields A, *et al.* Systemic infusion versus bolus chemotherapy with 5-fluorouracil in measurable metastatic colorectal cancer. *Am J Clin Oncol* 1992, **15(6)**, 518–523.
10. Rougier Ph, Paillot B, Laplanche A, *et al.* End results of a multicentric randomized trial comparing 5 FU in continuous systemic infusion (CI) to bolus administration (B) in measurable metastatic colorectal cancer (MMC). *Proc Am Soc Clin Oncol* 1992, **11**, 163 (abstract).
11. Arbuck SG. Overview of clinical trials using 5-fluorouracil and leucovorin for the treatment of colorectal cancer. *Cancer* 1989, **63**, 1036–1044.
12. Bruckner HW, Motwani BT. Chemotherapy of advanced cancer of the colon and rectum. *Semin Oncol* 1991, **18**, 443–461.
13. Piedbois P, Buyse M, Rustum Y, *et al.* Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. *J Clin Oncol* 1992, **10**, 896–903.
14. Hines JD, Adelstein DJ, Spiess JL, Giroski P, Carter SG. Efficacy of high-dose oral leucovorin and 5-fluorouracil in advanced colorectal carcinoma. *Cancer* 1989, **63**, 1022–1025.
15. Laufman LR, Brenckman WD, Stydnicki KA, *et al.* Clinical experience with leucovorin and 5-fluorouracil. *Cancer* 1989, **63**, 1031–1035.
16. Laufman LR, Bukowski RM, Collier MA, *et al.* A randomized, double-blind trial of fluorouracil plus placebo versus fluorouracil plus oral leucovorin in patients with metastatic colorectal cancer. *J Clin Oncol* 1993, **11**, 1888–1893.
17. Sinnige HAM, Buter J, De Vries EGE, *et al.* Phase I–II study of the addition of α-2a interferon to 5-fluorouracil/leucovorin. Pharmacokinetic interaction of α-2a interferon and leucovorin. *Eur J Cancer* 1993, **29A**, 1715–1720.
18. Hansen R, Beatty P, Quebbeman E, Anderson T. Continuous 5-fluorouracil (5FU) infusion and oral calcium leucovorin (CLV). A phase I trial. *Proc Am Soc Clin Oncol* 1991, **10**, 157 (abstract).
19. Tempero M, Berg A, Block M, Hutchins M, Moravec D, Langdon R. A phase II trial of protracted therapy with 5-fluorouracil and leucovorin in metastatic colorectal cancer. *Proc Am Soc Clin Oncol* 1992, **11**, 189 (abstract).
20. Poorter RL, Bakker PJM, Taat CW, Veenhof CHN. Intermittent continuous intravenous infusion of 5-fluorouracil; a useful approach in disseminated colorectal cancer? *Eur J Cancer* 1993, **29A**, 775.
21. World Health Organization (WHO). *WHO Handbook for Reporting Results of Cancer Treatment*. WHO, 1985.
22. Van Groeningen CJ, Pinedo HM, Heddes J, *et al.* Pharmacokinetics of 5-fluorouracil assessed with a sensitive mass spectrometric method in patients during a dose escalation schedule. *Cancer Res* 1988, **48**, 6956–6961.
23. Peters BJ, Lankelma J, Kok RM, *et al.* Prolonged retention of high concentrations of 5-fluorouracil in human and murine tumors as compared with plasma. *Cancer Chemother Pharmacol* 1993, **31**, 269–276.
24. Kok RM, De Jong APJM, Van Groeningen CJ, *et al.* Highly sensitive determination of 5-fluorouracil in human plasma by capillary gas chromatography and negative ion chemical ionization mass spectrometry. *J Chromatogr* 1985, **343**, 59–66.
25. Trave F, Rustum YM, Petrelli NJ, *et al.* Plasma and tumor tissue pharmacology of high-dose intravenous leucovorin calcium in combination with fluorouracil in patients with advanced colorectal carcinoma. *J Clin Oncol* 1988, **6**, 1184–1191.
26. Harris BE, Song R, Soong S, *et al.* Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990, **50**, 197–201.
27. Codacci-Pisanelli G, Van der Wilt CL, Pinedo HM, *et al.* Antitumour activity, toxicity and inhibition of thymidylate synthase of prolonged administration of 5-fluorouracil in mice. *Eur J Cancer* 1995, **31A**, 1517–1525.
28. Straw JA, Szapary D, Wynn WT. Pharmacokinetics of the diastereoisomers of leucovorin after intravenous and oral administration to normal subjects. *Cancer Res* 1984, **44**, 3114–3119.
29. Lokich JJ, Moore C. Chemotherapy-associated palmar-plantar erythrodyesthesia syndrome. *Ann Intern Med* 1984, **101**, 798–800.
30. Peters GJ, Codacci-Pisanelli G, Van der Wilt CL, *et al.* Comparison of continuous infusions and bolus injections of 5-fluorouracil with or without leucovorin; implications for inhibition of thymidylate synthase. In Rustum YM, ed. *Novel Approaches to Selective Treatments of Human Solid Tumors: Laboratory and Clinical Correlation*. New York, Plenum Press, 1993, 9–20.
31. Freeman NJ, Constanza ME. 5-Fluorouracil-associated cardiotoxicity. *Cancer* 1988, **61**, 36–45.
32. Labianca R, Beretta G, Clerici M, Fraschini P, Luporini G. Cardiac toxicity of 5-fluorouracil: a study on 1083 patients. *Tumori* 1982, **68**, 505–510.

33. Peters GJ, Van der Wilt CL, Van Groeningen CJ. Predictive value of thymidylate synthase and dihydropyrimidine dehydrogenase. *Eur J Cancer* 1994, **30A**, 1408–1411.
34. Van der Wilt CL, Peters GJ. New targets for pyrimidine antimetabolites in the treatment of solid tumours. 1. Thymidylate synthase. *Pharm World Sci* 1994, **16**, 84–103.
35. Evans RM, Laskin JD, Hakala MT. Effect of excess folates and deoxyinosine on the activity and site of action of 5-fluorouracil. *Cancer Res* 1981, **41**, 3288–3295.
36. Keyomarsi K, Moran RG. Folinic acid augmentation of the effects of fluoropyrimidines on murine and human leukemic cells. *Cancer Res* 1986, **46**, 5229–5235.
37. Etienne MC, Milano G, Lagrange JL, *et al.* Marked fluctuations in drug plasma concentrations caused by use of portable pumps for fluorouracil continuous infusion. *J Natl Cancer Inst* 1993, **85**, 1005–1007.
38. Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil. *Clin Pharmacokinet* 1989, **16**, 215–237.
39. Hillcoat BL, McCulloch PB, Figueredo AT, Ehsan MH, Rosenfeld JM. Clinical response and plasma levels of 5-fluorouracil in patients with colonic cancer treated by drug infusion. *Br J Cancer* 1978, **38**, 719–724.
40. Presant CA, Wolf W, Waluch V, *et al.* Association of intratumoral pharmacokinetics of fluorouracil with clinical response. *Lancet* 1994, **343**, 1184–1187.