Cancer Chemother Pharmacol (1994) 35: 144–148

ORIGINAL ARTICLE

Jan H. M. Sips · Olaf van Tellingen · Willem J. Nooijen
Sjoerd Rodenhuis · Wim W. Ten Bokkel Huinink
Jos H. Beijnen

The pharmacokinetics of reduced folates after intraperitoneal and intravenous administration of racemic [6S,R]-folinic acid

Received: 15 January 1994 / Accepted: 23 May 1994

Abstract We investigated the pharmacokinetics of tetrahydrofolates following the administration of [*6S,R*]-folinic acid and 5-fluorouracil delivered i.v., i.p., and by a combination of both routes in patients with colon cancer. The concentrations of the biologically active tetrahydrofolates ([*6S*]-folinic acid and 5-methyltetrahydrofolate) and the relatively inert diastereomer [*6R*]-folinic acid were monitored using a selective on-line coupled achiral-chiral high-performance liquid chromatographic method. In plasma, a target concentration of 5 μ*M* active tetrahydrofolates, which is considered necessary for an optimal synergistic effect, could be achieved after i.v. or combined i.v. and i.p. administration but was not reached in a patient receiving i.p. [*6S,R*]-folinic acid alone. In three patients receiving i.p. [*6S,R*]-folinic acid a high level of [*6S*]-folinic acid was observed in ascites, suggesting that the peritoneal cavity may act as a storage site for tetrahydrofolates after i.p. administration. In these patients, only a trace level of the active metabolite 5-methyltetrahydrofolate was detected in ascites, which may indicate that tetrahydrofolate derivatives penetrate only minimally, if at all, into the peritoneal cavity from the central compartment. These data would indicate that a combination of i.p. and i.v. administration may, from the pharmacological point of view, indeed contribute to an improved treatment of minimal residual disease persisting in the peritoneal cavity.

J. H. M. Sips · O. van Tellingen (✉) · W. J. Nooijen
Department of Clinical Chemistry, Antoni van Leeuwenhoek Huis/The Netherlands Cancer Institute,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

J. H. M. Sips · J. H. Beijnen
Department of Pharmacy, Slotervaart Hospital, Louwesweg 6,
1066 EC Amsterdam, The Netherlands

S. Rodenhuis · W. W. Ten Bokkel Huinink
Division of Medical Oncology, Antoni van Leeuwenhoek Huis/The Netherlands Cancer Institute,
Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Key words Pharmacokinetics · Intraperitoneal administration · Folates · Stereoselectivity · High-performance liquid chromatography

Introduction

[*6S,R*]-Folinic acid (5-formyltetrahydrofolate, leucovorin; [*6S,R*]-LV) is a reduced folate derivative that has been used for more than 20 years as a rescue agent for high-dose methotrexate therapy [1]. During the last decade, several laboratory and clinical studies have demonstrated that the cytotoxic effect of 5-fluorouracil (5-FU) is substantially potentiated in the presence of sufficient amounts of reduced folates [16, 25, 34]. The biochemical rationale for this synergism is a stabilization of the ternary complex formed between the target enzyme thymidylate syntethase, the activated form of 5-FU, 5-fluorodeoxyuridylate (5FdUMP), and 5,10-methylenetetrahydrofolate (5,10-methyleneTHF), resulting in a more efficient depletion of thymidylate [8, 13, 36]. The cofactor 5,10-methyleneTHF is formed intracellularly from other reduced folate derivatives. Although some uncertainty exists about the target concentration of reduced folates in plasma, most in vitro studies performed in murine or human tumor cell lines indicate that a concentration of 5 μ*M* is sufficient for a maximal synergistic effect [10, 14, 22]. This synergistic concept has been studied in several clinical trials in patients with advanced colorectal [15, 17, 21, 25], breast [9, 26, 34], and head and neck cancer [37].

Commercially available [*6S,R*]-LV consists of equal amounts of the two diastereomers [*6S*]-LV and [*6R*]-LV, whereas the stereochemical configuration at the C-6 position of the naturally occurring folates (e.g., 5,10-methyleneTHF, 5-methylTHF) is always similar to that of [*6S*]-LV. The first published methods for the determination of [*6S*]-LV are bioassays based on the dependence of bacterial cultures on folates as a growth factor [19, 20]. Although such methods are sensitive, they are generally not selective for one specific folate. Both Hamel et al. [12] and Bunni et

Table 1 Summary of the pharmacokinetics of tetrahydrofolates ($t > 5$ μM Time for which the concentration of active folates, i.e., [6*S*]-LV + 5-methylTHF, exceeds the target level at 5 μ*M*)

Patient number	Route (dose)[a]	[6*R*]-LV AUC (μM h)	$t_{1/2}$ (h)	Cl (l h^{-1} m^{-2})	C_{max} (μ*M*)	[6*S*]-LV AUC (μM h)	$t_{1/2}$ (h)	Cl (l h^{-1} m^{-2})	C_{max} (μ*M*)	5-methylTHF AUC (μM h)	$t_{1/2}$ (h)	C_{max} (μ*M*)	t > 5 μ*M* (h)
1	i.v. (80)	297	15.0	0.135	19.6	11.9	0.93	3.35	7.4	46.9	8.0	3.6	4
2		133	6.3	0.301	16.0	13.0	0.48	3.09	10.0	19.3	3.7	2.8	2
3	i.p. (80)	162	8.8	0.247	10.2	6.6	1.03	6.03	2.0	32.0	8.5	2.0	0
4	i.p./i.v.	227	6.0	0.353	14.0	21.4	1.03	3.74	8.6	47.9	4.1	5.8	6
5	(80/80)	482	11.8	0.165	29.2	35.1	1.25	2.27	14.6	70.1	7.2	5.0	8

[a] Dose level in mg/m^2

al. [6] have described more selective radioenzymatic assays, but the relative complexity of these methods and the difficulty in obtaining the necessary reagents make these assays less suitable for routine monitoring and pharmacokinetics studies. High-performance liquid chromatography (HPLC) is a widely used technique for the determination of folates and antifolates in biological fluids. Commonly used reversed-phase systems, however, cannot differentiate between [6*S*]-LV and [6*R*]-LV [2, 4]. Straw et al. [30] circumvented this drawback by combining an achiral HPLC method with a bioassay to estimate the amount of [6*S*]-LV in the LV peak containing both diasteriomers. More recently, with the introduction of chiral HPLC columns, a number of papers dealing with the bioanalysis of both LV diastereomers and the major metabolic product 5-methylTHF have been published [7, 27, 28, 38]. Clinical pharmacokinetics studies with oral or i.v. [6*S,R*]-LV have shown a substantial difference in pharmacokinetic behavior between the two diastereomers [18, 23, 27, 28, 31]. These results emphasize the need for using stereoselective analytical methods to describe the pharmacokinetic profiles.

To obtain a well-tolerated chemotherapy regimen suitable for the palliation of intractable ascites or for the adjuvant treatment of high-risk colon cancer, a weekly regimen consisting of both i.v. and i.p. 5-FU and [6*S,R*]-LV was developed in our institute. The present pharmacokinetics study was undertaken to monitor whether the target level of 5 μ*M* active folates ([6*S*]-LV plus 5-methylTHF) could be achieved with this regimen. The concentrations of [6*S*]-LV, 5-methylTHF, and the inactive [6*R*]-LV in both plasma and ascites were determined using an on-line coupled achiral-chiral HPLC system by analogy with the method described by Wainer and Stiffin [38]. The achiral HPLC system is used to separate [6*S,R*]-LV from 5-methylTHF and to quantify both compounds selectively by UV detection at 305 nm. The column effluent containing the [6*S,R*]-LV peak was directed by means of a switching valve onto a chiral bovine serum albumin column for separation and determination of the ratio of the two diasteromers.

Materials and methods

Reagents

[6*S,R*]-LV was purchased from Holland Pharmaceutical Supply BV (Alphen aan de Rijn, The Netherlands) and 5-methylTHF was obtained from Sigma (St. Louis, USA). All other reagents were supplied by E. Merck (Darmstadt, Germany) and were of analytical quality.

HPLC analysis

Our previously described reversed-phase HPLC method for the determination of 5-methylTHF and [6*S,R*]-LV [35] was slightly adapted to ensure compatibility with the secondary chiral HPLC system. In short, the achiral HPLC system consisted of two Spectoflow SF400 pumps, a Spectroflow SF757 UV/VIS detector operating at 305 nm (Kratos, Ramsey, USA), a Model 5140 Solvent Programmer (Kipp & Zonen, Delft, The Netherlands), a Model 360 Autosampler (Kontron Instruments, Basel, Switzerland) provided with a 500-μl sample loop, and a Model SP4270 dual-channel integrator (Spectra-Physics, San Jose, USA). Further data analysis was performed by WINner/286 software (Spectra-Physics).

Achiral chromatographic separation was achieved on a glass column (200×3 mm inside diameter) packed with 5-μm Hypersil-ODS material and protected by a guard column (10×3 mm) packed with pellicular RP material (Chrompack, Middelburg, The Netherlands). Solvent A consisted of 25 m*M* potassium biphosphate buffer adjusted to pH 3.5 with hydrochloric acid. Solvent B consisted of 10% 1-propanol in solvent A. The flow rate was maintained at 0.4 ml/min and the column was kept at 40° C. The starting condition was 90% solvent A and 10% solvent B. At time zero a 10-min linear gradient from 10% to 40% solvent B was started, followed by 1 min with 40% solvent B, 3 min at 100% solvent B, and 15 min reequilibration at 10% solvent B before the next injection. A MUST switching valve (Sparks Holland, Emmen, The Netherlands) provided with an 800-μl sample loop was placed immediately after the SF757 UV detector. At a timed event the eluate fraction containing [6*S,R*]-LV is switched onto a Revolvosil BSA-7 column (150×4 mm inside diameter; Macherey Nagel, Düren, Germany) protected by a Revolvosil guard column (30×4 mm) and thermostated at 40° C. The mobile phase consisted of 0.2 *M* potassium phosphate buffer (pH 5.0) and was pumped at a flow rate of 1.0 ml/min by a SF400 solvent delivery system provided with a high-efficiency membrane pulse damper (Kratos). Detection was performed at 285 nm using a 1000S Photo diode-array detector (Kratos).

Sample preparation

A volume of 500 μl ice-cold plasma, 50 μl ascorbic acid solution (10 g/l) and 500 μl ice-cold perchloric acid (1.5 *M*) was vortex-mixed

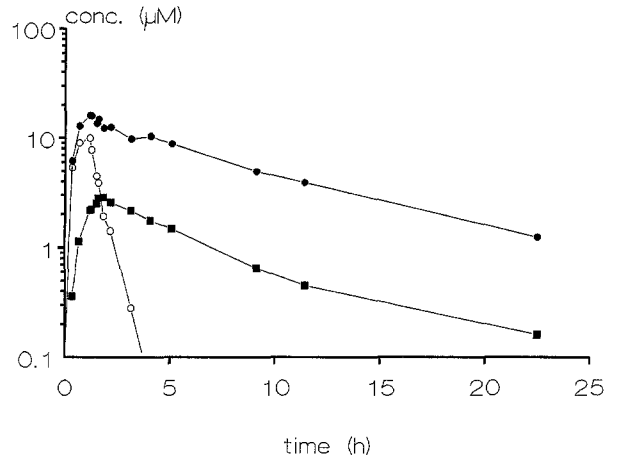

Fig. 1 Concentration versus time curves generated for tetrahydrofolates in plasma following the i.v. administration of 80 mg/m². –○–, [*6S*]-LV; –●–, [*6R*]-LV; –■–, 5-methylTHF

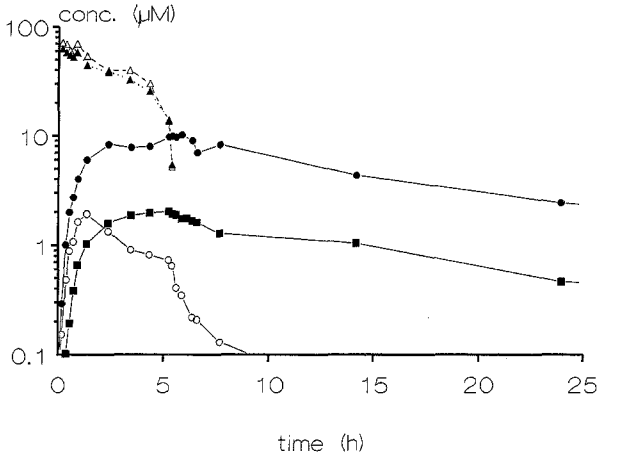

Fig. 2 Concentration versus time curves generated for tetrahydrofolates in plasma and ascites after i.p. administration of 80 mg/m². –○–, [*6S*]-LV in plasma; –△–, [*6S*]-LV in ascites; –●–, [*6R*]-LV in plasma; –▲–, [*6R*]-LV in ascites; –■–, 5-methylTHF in plasma

and placed on ice for 5 min. Next, the sample was centrifuged (5 min, 3000 *g*, 4° C) and an aliquot of 700 µl of the supernatant fluid was vortex-mixed with 100 µl potassium acetate solution (8 *M*). After a 2-min incubation in ice water the sample was centrifuged (2 min, 3000 *g*, 4° C) and 500 µl of the supernatant fluid was mixed with 50 µl ascorbic acid solution (100 g/l). An aliquot of 500 µl was subjected to chromatography.

Clinical regimens of [6S,R]-LV administration

Five patients with colorectal cancer participated in this kinetics study. Patients 1 and 2 received a 1-h i.v. infusion of 80 mg/m² [*6S,R*]-LV immediately followed by an i.v. bolus injection of 400 mg/m² 5-FU. Patient 3 received 80 mg/m² [*6S,R*]-LV and 400 mg/m² 5-FU, which was dissolved in 2 l dialysis fluid (Dianeal) and instilled into the peritoneal cavity with a dwell time of 4 h. The runout time amounted to approximately 1 h. Patients 4 and 5 received the same i.p. regimen; however, the dialysis fluid remained in the peritoneal cavity. At 1 h after the start of the i.p. administration, these patients also received a 1-h i.v. infusion with 80 mg/m² [*6S,R*]-LV followed by an i.v. bolus of 400 mg/m² 5-FU.

Blood (patients 1–5) and ascites (patient 3–5) samples were drawn at various intervals between 0 and 24 h. Blood samples (5 ml) were collected in heparinized tubes containing approximately 10 mg ascorbic acid. Plasma was separated by centrifugation (1500 *g*, 10 min) and was stored immediately at –20° C. Ascites (5 ml) was collected in ethylenediaminetetraacetic acid (EDTA)-coated tubes also containing 10 mg ascorbic acid and was stored immediately at –20° C.

Pharmacokinetic analysis

The plasma area under the curve (AUC) for all folates was calculated using the linear trapezoidal rule with extrapolation to infinity. The terminal half-lives ($t_{1/2}$) were estimated from the final linear part of the log-linear concentration-time curves, and the plasma clearnace (Cl) was calculated by the formula $Cl = Dose/AUC$.

Results

The plasma pharmacokinetic parameters are summarized in Table 1. Following the i.v. administration of racemic [*6S,R*]-LV, [*6S*]-LV levels declined much faster than [*6R*]-LV levels. The rapid decrease in [*6S*]-LV levels was accompanied by the rapid appearance of 5-methylTHF (Fig. 1). The target level of 5 µ*M* active folates ([*6S*]-LV plus 5-methylTHF) was exceeded for 2–4 h.

After i.p. administration, both [*6S*]-LV and [*6R*]-LV were equally well absorbed from the peritoneal cavity (Fig. 2), with approximately 40% of the dose being recovered in the tap fluid after a dwell time of 4 h. During this time the i.p. levels remained far higher than 5 µ*M*, with no substantial difference in the ratio of the diastereomers being found. Only trace levels of 5-methylTHF were detected in the peritoneal fluid. Almost immediately after i.p. administration, both folates emerged in plasma and the kinetic profiles were similar to those seen after i.v. administration. A plasma level of 5 µ*M* active folates was not achieved during any period within the investigated interval of 24 h.

After combined i.p. plus i.v. administration, high plasma levels (with concentrations of active folates exceeding 5 µ*M*) were present for a period of 6–8 h (Fig. 3). In ascites, approximately 4–5 h after the start of the i.p. administration, a change in the ratio of the two diastereomers was noted, with [*6S*]-LV concentrations declining more rapidly than [*6R*]-LV levels. Also after combined i.v. plus i.p. treatment, only trace levels of 5-methylTHF were detected in ascites.

Discussion

Both ovarian cancer and gastrointestinal malignancies tend to spread through the peritoneal cavity, forming large numbers of implantation metastases ("peritoneal carcinosis"). Development of i.p. administration of cytotoxic drugs may improve the palliative treatment of patients with these malignancies, due to a more intensive i.p. drug exposure combined with diminished systemic exposure. 5-FU

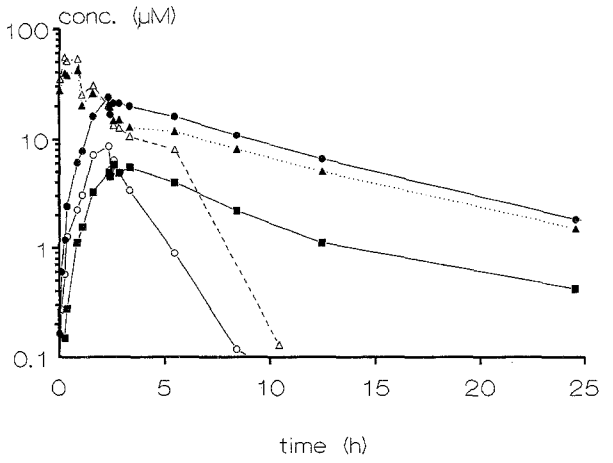

Fig. 3 Concentration versus time curves generated for tetrahydrofolates in plasma and ascites after i.p. administration of 80 mg/m² followed by i.v. administration of 80 mg/m² after 1 h. –○–, [6*S*]-LV in plasma; –△–, [6*S*]-LV in ascites; –●–, [6*R*]-LV in plasma; –▲–, [6*R*]-LV in ascites; –■–, 5-methylTHF in plasma

given i.p. has been used to treat ovarian and gastrointestinal cancer [11, 24, 29, 33], whereas the i.p. combination of 5-FU with [6*S,R*]-LV has been reported by Budd et al. [5] and Arbuck et al. [3]. The latter study included data on folate kinetics, but the dose levels used were too low to achieve a target level of 5 μ*M* active folates in plasma. The present pharmacokinetics study was undertaken to increase our insight into folate kinetics so as to substantiate the pharmacological basis of possible future clinical studies.

The present investigation and other studies show that independent of the route of administration, the diastereomers differ substantially in their pharmacokinetic behavior, with [6*S*]-LV being eliminated from plasma more rapidly than [6*R*]-LV. These observations can be explained by the stereoselectivity of the transport proteins and enzymes involved in the pathways of folate biochemistry. [6*S*]-LV is transported freely into cells and metabolized to other tetrahydrofolate derivatives, whereas [6*R*]-LV is a relatively inert compound and is, for the major part, excreted unchanged in the urine [28, 31]. Because the plasma half-life of 5-methylTHF is longer than that of [6*S*]-LV, a few hours after administration this compound is the most prevalent tetrahydrofolate in plasma. Since the donating potency of this molecule as a cofactor in 1-carbon metabolism is minimal, the abundant formation of this compound can be conceived as the generation of a deposit for the surplus of tetrahydrofolates, in particular because interconversion to more active cofactors such as 5,10-methyleneTHF can occur rapidly when necessary [32]. Due to the presence of such abundant amounts of 5-methylTHF, tissues will be saturated and the elimination of this compound will be determined mostly by excretion and only minimally, if at all, by the consumption effected by its distribution and/or metabolism.

When we used the conventional therapy with i.v. administration of 80 mg/m² [6*S,R*]-LV as a 1-h infusion, a systemic plasma level of active folates higher than 5 μ*M* was obtained for a period of 2–4 h. Unfortunately, collection of ascites was not possible in these patients. High i.p. levels were attained following i.p. administration of the same dose dissolved in 2 l dialysis fluid (approx. 75 μ*M* [6*S*]-LV. However, the target level of 5 μ*M* in plasma was not achieved in the patient who received i.p. folinic acid alone, and this situation was considered undesirable since it indicated a possibly less efficacious treatment of extraperitoneal disease. A combination of i.p. plus i.v. treatment provided the desired target levels of active folates in both plasma and ascites, which were sustained for a considerable period.

The results obtained in the two patients who received [6*R,S*]-LV by i.p. and i.v. administration and in whom the instilled fluid remained in the peritoneal cavity illustrate that this compartment acts as a storage pool for both diastereomers and that the rate of disappearance from the i.p. cavity of each is dictated by its elimination rate from plasma. In ascites the ratio of [6*S*]-LV to [6*R*]-LV remained constant until peak levels were reached in plasma (Fig. 3). Next, the decline of i.p. [6*R*]-LV closely paralleled that of [6*R*]-LV in plasma. Due to its higher plasma clearance the supply of [6*S*]-LV in peritoneal fluid is depleted more rapidly, although the transfer of this compound from the peritoneal cavity appears to be rate-limiting.

After i.p. or i.v. plus i.p. administration, only trace levels of 5-methylTHF were detected in ascites, indicating that tetrahydrofolates cannot penetrate from the central compartment into he peritoneal cavity to such an extent that effective i.p. concentrations can be expected. Consequently, the i.p. cavity appears to behave like a pharmacological sanctuary, and only low levels of active folates may be expected in ascites when [6*S,R*]-LV is given systemically (i.v. or orally). This would suggest that a combination of i.p. and i.v. administration may, from a pharmacological point of view, indeed contribute to an improved treatment of minimal residual disease persisting in the peritoneal cavity.

References

1. Ackland SP, Schilsky RL (1987) High-dose methotrexate: a critical reappraisal. J Clin Oncol 5: 2017–2031
2. Allen BA, Newman RA (1980) High-performance liquid chromatographic separation of clinically important folic acid derivatives using ion-pair chromatography. J Chromatogr 190: 241–245
3. Arbuck SG, Trave F, Douglass HO Jr, Nava H, Zakrezewski S, Rustum YM (1986) Phase I and pharmacologic studies of intraperitoneal leucovorin and 5-fluorouracil in patients with advanced cancer. J Clin Oncol 4: 1510–1517
4. Birmingham BK, Greene DS (1983) Analysis of folinic acid in human serum using high-performance liquid chromatography with amperometric detection. J Pharm Sci 72: 1306–1309
5. Budd GT, Schreiber MJ, Steiger E, Bukowski RM, Weick JK (1986) Phase I trial of intraperitoneal chemotherapy with 5-fluorouracil and citrovorum factor. Invest New Drugs 4: 155–158
6. Bunni MA, Rembiesa BM, Priest DG, Sahovic E, Stuart R (1989) Accumulation of tetrahydrofolates in human plasma after leucovorin administration. Cancer Chemother Pharmacol 23: 353–357

7. Choi KE, Schilsky RL (1988) Resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate by chiral high-performance liquid chromatography. Anal Biochem 168: 398–404
8. Danenberg PV, Danenberg KD (1978) Effect of 5,10-methylenetetrahydrofolate on the dissociation of 5-fluoro-2′-deoxyuridylate from thymidylate synthetase: evidence for an ordered mechanism. Biochemistry 17: 4018–4024
9. Doroshow JH, Leong L, Margolin K, Flanagan B, Goldberg D, Bertrand M, Akman S, Carr B, Odujinrin O, Newman E (1989) Refractory metastatic breast cancer: salvage therapy with fluorouracil and high-dose continuous infusion leucovorin calcium. J Clin Oncol 7: 439–444
10. Evans RM, Laskin JD, Hakala MT (1981) Effect of excess folates and deoxyinosine on the activity and site of action of 5-fluorouracil. Cancer Res 41: 3288–3295
11. Gyves JW, Ensminger WD, Stetson P (1984) Constant intraperitoneal 5-fluorouracil infusion through a totally implanted system. Clin Pharmacol Ther 35: 83–89
12. Hamel E, Johnson G, Glaubiger D (1981) Pharmacokinetics of leucovorin rescue using a new methotrexate-independent biochemical assay for leucovorin and *N*5-methyltetrahydrofolate. Cancer Treat Rep 65: 545–553
13. Houghton JA, Schmidt C, Houghton PJ (1982) The effect of derivatives of folic acid on the fluorodeoxyuridylate-thymidylate synthetase covalent complex in human colon xenografts. Eur J Cancer Clin Oncol 18: 347–354
14. Keyomarsi K, Moran RG (1986) Folinic acid augmentation of the effects of fluoropyrimidines on murine and human leukemic cells. Cancer Res 46: 5229–5235
15. Laufman LR, Brenckman WD Jr, Stydnicki KA, Morgan ED, Collier M, Knick VB, Duch DS, Mullin R, Ferone R (1989) Clinical experience with leucovorin and 5-fluorouracil. Cancer 63: 1031–1035
16. Machover D, Goldschmidt E, Chollet P, Metzger G, Zittoun J, Marquet J, Vandenbulcke JM, Misset JL, Schwarzenberg L, Fourtillan JB (1986) Treatment of advanced colorectal and gastric adenocarcinomas with 5-fluorouracil and high-dose folinic acid. J Clin Oncol 4: 685–696
17. Madajewics S, Petrelli N, Rustum YM, Campbell J, Herrera L, Mittelman A, Perry A, Creaven PJ (1984) Phase I-II trial of high-dose calcium leucovorin and 5-fluorouracil in advanced colorectal cancer. Cancer Res 44: 4667–4669
18. McGuire BW, Sia LL, Haynes JD, Kisicki JC, Gutierrez ML, Stokstad EL (1987) Absorption kinetics of orally administered leucovorin calcium. Natl Cancer Inst Monogr 5: 47–56
19. Mehta BM, Hutchison DJ (1975) Assay for citrovorum factor (NSC-3590) in the presence of methotrexate (NSC-740). Cancer Chemother Rep 59: 935–937
20. Mehta BM, Gisolfi AL, Hutchison DJ, Nirenberg A, Kellick MG, Rosen G (1978) Serum distribution of citrovorum factor and 5-methyltetrahydrofolate following oral and i.m. administration of calcium leucovorin in normal adults. Cancer Treat Rep 62: 345–350
21. Mini E, Trave F, Rustum YM, Bertino JR (1990) Enhancement of the antitumor effects of 5-fluorouracil by folinic acid. Pharmacol Ther 47: 1–19
22. Moran RG (1989) Leucovoring enhancement of the effects of the fluoropyrimidines on thymidylate synthase. Cancer 63: 1008–1012
23. Newman EM, Straw JA, Doroshow JH (1989) Pharmacokinetics of diastereoisomers of (6R,S)-folinic acid (leucovorin) in humans during constant high-dose intravenous infusion. Cancer Res 49: 5755–5760
24. Ozols RF, Speyer JL, Jenkins J, Myers CE (1984) Phase II trial of 5-FU administered i.p. to patients with refractory ovarian cancer. Cancer Treat Rep 68: 1229–1232
25. Petrelli N, Douglass HO Jr, Herrera L, Russell D, Stablein DM, Bruckner HW, Mayer RJ, Schinella R, Green MD, Muggia FM (1989) The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. Gastrointestinal Tumor Study Group [published erratum appears in J Clin Oncol 8: 185]. J Clin Oncol 7: 1419–1426
26. Pronzato P, Amoroso D, Ardizzoni A, Bertelli G, Canobbio L, Conte PF, Cusimano MP, Fusco V, Gulisano M, Lionetto R (1987) Sequential administration of cyclophosphamide, methotrexate, 5-fluorouracil, and folinic acid as salvage treatment in metastatic breast cancer. Am J Clin Oncol 10: 404–406
27. Schalhorn A, Kuhl M, Stupp Poutot G, Nussler V (1990) Pharmacokinetis of reduced folates after short-term infusion of *d,l*-folinic acid. Cancer Chemother Pharmacol 25: 440–444
28. Schilsky RL, Ratain MJ (1990) Clinical pharmacokinetics of high-dose leucovorin calcium after intravenous and oral administration. J Natl Cancer Inst 82: 1411–1415
29. Speyer JL, Collins JM, Dedrick RL, Brennan MF, Buckpitt AR, Londer H, DeVita VT, Myers Ce (1980) Phase I and pharmacological studies of 5-fluorouracil administered intraperitoneally. Cancer Res 40: 567–572
30. Straw JA, Szapary D, Wynn WT (1984) Pharmacokinetics of the diastereoisomers of leucovorin after intravenous and oral administration to normal subjects. Cancer Res 44: 3114–3119
31. Straw JA, Newman EM, Doroshow JH (1987) Pharmacokinetics of leucovorin (D,L-5-formyltetrahydrofolate) after intravenous injection and constant intravenous infusion. Natl Cancer Inst Monogr 5: 41–45
32. Stryer L (1981) Biochemistry, 2nd edn. W. H. Freeman, New York, pp 491–494
33. Sugarbaker PH, Gianola FJ, Speyer JL, Wesly R, Barofski I, Myers Ce (1985) Prospective randomized trial of intravenous v intraperitoneal 5-FU in patients with a primary colon or rectal cancer. Semin Oncol 12: 101–111
34. Swain SM, Lippman ME, Egan EF, Drake JC, Steinberg SM, Allegra CJ (1989) Fluorouracil and high-dose leucovorin in previously treated patients with metastatic breast cancer. J Clin Oncol 7: 890–899
35. Tellingen O van, Woude HR van der, Beijnen JH, Beers CJ van, Nooijen WJ (1989) Stable and sensitive method for the simultaneous determination of *N*5-methyltetrahydrofolate, leucovorin, methotrexate and 7-hydroxymethotrexate in biological fluids. J Chromatogr 488: 379–388
36. Ullman B, Lee M, Martin DW Jr, Santi DV (1978) Cytotoxicity of 5-fluoro-2′-deoxyuridine: requirement for reduced folate cofactors and antagonism by methotrexate. Proc Natl Acad Sci USA 75: 980–983
37. Vokes EE, Choi Ke, Schilsky RL, Moran WJ, Guarnieri CM, Weichselbaum RR, Panje WR (1988) Cisplatin, fluorouracil, and high-dose leucovorin for recurrent or metastatic head and neck cancer. J Clin Oncol 6: 618–626
38. Wainer IW, Stiffin RM (1988) Direct resolution of the stereoisomers of leucovorin and 5-methyltetrahydrofolate using a bovine serum albumin high-performance liquid chromatographic chiral stationary phase coupled to an achiral phenyl column. J Chromatogr 424: 158–162