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Role of Leucovorin Dosing and Administration Schedule

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Leucovorin (LV) is commonly administered in association with 5-fluorouracil (5-FU) to enhance its cytotoxic effects. In this paper, the cellular and clinical pharmacology of 5-FU potentiation by LV are reviewed, and the dosing and administration schedules are discussed in relation to reported clinical trials. *In vitro* experimental data suggest that prolonged cellular exposures to relatively low LV concentrations simultaneously with prolonged 5-FU administration are the optimal conditions to enhance 5-FU efficacy. Clinical studies of 5-FU/LV in metastatic colorectal carcinoma have established that 5-day bolus 5-FU with low-dose bolus LV injections yield therapeutic benefits equivalent to those obtained with intravenous bolus schedules using higher doses of LV. It remains to be determined, however, if bolus administration schedules are the optimal clinical treatment regimens. Infusional 5-FU/LV regimens appear to be a strategy worthy of further clinical investigation.

Key words: leucovorin, 5-fluorouracil, pharmacology, colon cancer
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INTRODUCTION

5-FORMYLTETRAHYDROFOLATE MONOGLUTAMATE (5-formyl-H_4folate) is administered clinically under the trade name leucovorin (LV) in conjunction with chemotherapeutic agents. While initially used to rescue normal cells from the toxic effects of high-dose antifolate therapy [1], LV is now more commonly administered in association with 5-fluorouracil (5-FU) to enhance its cytotoxic effects [2]. The combination of 5-FU and LV has been extensively tested clinically, and has clearly been shown to be superior to 5-FU alone in the treatment of patients with metastatic colorectal carcinoma [3]. 5-FU/LV combinations are now commonly used to treat patients with metastatic colorectal carcinoma [4], and are being investigated as adjuvant therapy following primary resection of colon adenocarcinoma [5, 6]. In this paper, the cellular and clinical pharmacology of 5-FU-LV will be reviewed, and the importance of dosing and administration schedules discussed in relation to reported clinical trials.

THE CELLULAR PHARMACOLOGY OF 5-FU/LV INTERACTIONS

Folic acid, upon reduction to the tetrahydro form (H_4folate) by the enzyme dihydrofolate reductase, functions as a coenzyme for the transfer, oxidation and reduction of single carbon units [7]. Three of the one-carbon substituted derivatives of H_4folate are associated with particular metabolic cycles as shown in Figure 1: 5-methylH_4folate is involved in methionine synthesis, methyleneH_4folate in thymidylate synthesis and 10-formylH_4folate in purine synthesis. All folates are present in cells almost exclusively in the form of polyglutamates, folates to which γ-linked glutamic acid residues have been added to the terminal glutamyl moiety of folates by the enzyme folylpolyglutamate synthetase. Polyglutamylated folates have greater affinity for the folate-dependent enzymes than their monoglutamyl counterparts, and most of these enzymes are active *in vivo* only when the folate is in the polyglutamylated form [8]. Unsubstituted H_4folate is the preferred substrate for folate polyglutamate synthesis [9].

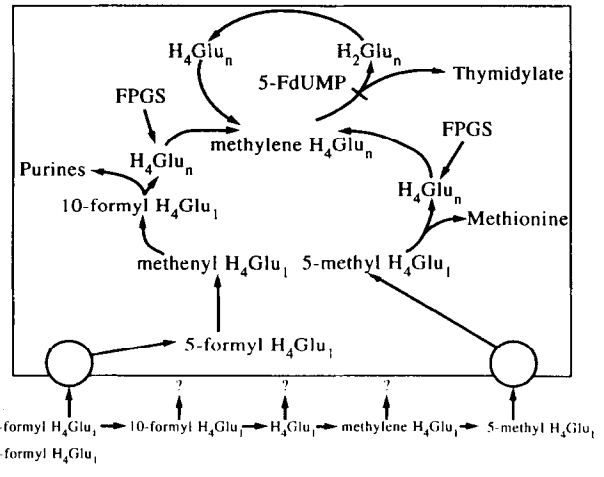

Figure 1. Metabolic cycles associated with leucovorin metabolism. H_4Glu_1, tetrahydrofolate monoglutamate; H_4Glu_n, tetrahydrofolate polyglutamates; 5-FdUMP, 5-fluorodeoxyuridylate monophosphate; FPGS, folylpolyglutamate synthetase.

5-FU's main site of action is thought to be the enzyme, thymidylate synthase, a protein which catalyses the transfer of the methylene group from methyleneH_4folate to deoxyuridylate to form thymidylate, a step essential for DNA synthesis

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(Figure 1). H_2folate is produced by this reaction and is regenerated to H_4folate via dihydrofolate reductase. The 5-FU metabolite 5-FdUMP inhibits thymidylate synthase activity as it forms a stable inactivating ternary complex with the enzyme and methyleneH_4folate [10]. Low methyleneH_4folate levels increase the ternary complex dissociation rate, leading to incomplete thymidylate synthase inhibition, and can be responsible for decreased fluoropyrimidine activity [11].

The cytotoxicity of 5-FU in several experimental models can effectively be enhanced by increasing intracellular folate concentrations through co-administration of fluoropyrimidines with LV [12, 13]. This product contains a mixture of the natural and unnatural diastereoisomers of 5-formylH_4folate with an S chirality at the 6 carbon of the tetrahydropterine ring [14], with the natural and unnatural isomers designated, respectively, (6S)- and (6R)-5-formylH_4folate (or L-and D-5-formylH_4folate). Although (6R)-5-formylH_4folate can be transported intracellularly and is a weak inhibitor of (6S)-5-formylH_4folate uptake, the unnatural isomer has no measurable impact on the natural isomer's replenishment of intracellular folate pools, support of cell growth or potentiation of 5-FU cytotoxicity *in vitro* [15–18].

(6S)-5-FormylH_4folate requires metabolism through four sequential enzymatic steps prior to transformation to methyleneH_4folate (Figure 1). In one study, low activity of the enzyme responsible for the initial metabolism of 5-formylH_4folate to methenylH_4folate, methyleneH_4folate synthetase, was associated with decreased intracellular folate interconversion to other folates in a rat tumour model [19]. 5-MethylH_4folate can also potentiate fluoropyrimidine toxicity *in vitro* [13], but must first be metabolised by vitamin B_{12}-dependent methionine synthase (Figure 1). In MCF-7 human breast cancer cells, methyleneH_4folate was detected intracellularly after 5-formylH_4folate, but not 5-methylH_4folate exposures [20], and 5-methylH_4folate has been shown to be less effective than 5-formylH_4folate in potentiating 5-FU activity in certain cell lines [21, 22].

MethyleneH_4folate polyglutamates containing three or more glutamyl residues bind more tightly to thymidylate synthase than do monoglutamates or diglutamates, increasing the stability of the ternary complex with 5-FdUMP [23]. The importance of this observation was demonstrated in a human CCRF-CEM human leukaemic cell subline with decreased folypolyglutamate synthetase activity [24]. Total intracellular folate levels and the pattern of folate polyglutamylation were similar before LV exposure in the parental cells and the synthetase deficient subline. However, after 2-h exposures to 10 μM LV, the parental cells contained primarily folate pentaglutamates and the subline contained mono- and diglutamates, resulting in significantly decreased fluoropyrimidine potentiation in the subline with decreased folypolyglutamate synthetase activity.

Despite prolonged exposures to up to 1000-fold elevations in extracellular LV concentrations, intracellular folate levels increase only 2–6-fold in many experimental systems [17, 21, 25–27]. In addition, as extracellular LV concentrations are increased, folates accumulate mostly as monoglutamates or short length polyglutamates [28], possibly because 5-formylH_4folate is a poor substrate for folylpolyglutamate synthetase [9].

Based on the available experimental data, it would appear that prolonged cellular exposures (≥ 24 h) to relatively low (μM) LV concentrations would be the optimal strategy to promote the formation of higher chain length folate polyglutamates [27].

LV PHARMACOKINETICS

Plasma pharmacokinetics of (6S)- and (6R)-5-formylH_4folate and 5-methylH_4folate have been examined after low-dose [29] and high-dose [30] intravenous, bolus and oral administration, and during high-dose intravenous infusions [31]. Following intravenous LV administrations, (6S)-5-formylH_4folate disappeared rapidly from plasma with mean elimination half-lives ranging from 30 to 60 min, and peak plasma concentrations of 2 and 60 μM following 50 and 1000 mg injections, respectively. During 500 mg/m^2/day LV infusions, plasma (6S)-5-formyl-H_4folate reached a steady-state concentration of approximately 3 μM. In all instances, (6R)-5-formylH_4folate had a slower clearance and accumulated to higher concentrations than the physiological stereoisomer for prolonged periods, and 5-methylH_4folate rapidly appeared in the plasma but, because of its slower clearance, persisted at a higher concentration than (6S)-5-formylH_4folate. Oral LV absorption was stereoselective after intake of 50 or 100 mg doses, with poor absorption of (6R)-5-formylH_4folate and little or no detectable natural isomer in the plasma, since first pass metabolism transformed it to 5-methylH_4folate, the main folate appearing in plasma.

Priest and coworkers re-examined LV plasma pharmacokinetics using a different methodology which also detects other one-carbon substituted reduced folates [32]. They have shown that both intravenous and oral LV administration, in addition to increased levels of 5-formylH_4folate and 5-methylH_4folate, resulted in a dose-dependent accumulation in the plasma of methyleneH_4folate, H_4folate and 10-formylH_4folate at micromolar concentrations. Erythrocyte metabolism appears to be a likely source for these metabolites [33], but their contribution to intracellular reduced folate pools remains to be determined. That assessment will have to take into account the plasma protein binding of the different reduced folate cofactors since recent *in vitro* studies have shown that (6R)-5-formylH_4folate was almost completely bound to albumin at physiological protein plasma concentrations, with 5-methylH_4folate 45% bound and (6S)-5-formylH_4folate not bound at all [34]. It thus seems likely that (6S)-5-formylH_4folate is a major contributor to intracellular folate replenishment after intravenous LV administration.

EXPERIMENTAL STUDIES OF 5-FU POTENTIATION BY LV

In vitro

In a recent review [35], LV was found to potentiate the cytotoxic effects of 5-FU *in vitro* in 41 out of 69 reported cell lines, usually with 2–3-fold increases in 5-FU activity. Potentiation of 5-FU cytotoxicity did not seem to be concentration-dependent above a threshold folate level of approximately 1 μM LV, and synergism was not clearly dependent on the sequence of LV and 5-FU exposures, with most investigators using simultaneous, prolonged (≥ 24 h) exposures. Moran and Scanlon determined, in two cell lines, that potentiation was not maximal until prolonged (≥ 24 h) exposures to both fluoropyrimidines and LV were used [36]. Significant decreases in 5-FU IC_{50} concentrations were found in 10 of 11 human colorectal carcinoma cell lines after 96-h simultaneous exposures to 5-FU and 20 μM LV [37]. The modest (usually ≤ 2-fold) level of potentiation was predicted to have a limited clinical impact, however, since three of the 11 human colon carcinoma cell lines tested were sensitive to 5-FU alone at clinically achievable drug concentrations, with only one additional line rendered sensitive following the addition of LV. In one of the largest reported single studies, 17 human cell lines were exposed for 5 days to

5-FU with or without LV in concentrations ranging from 0.0025 to 100 μM [38]. 5-FU potentiation up to 8-fold was seen in 12/17 cell lines, with optimal LV concentrations ≤ 5 μM in nine lines, with the other three requiring 13, 78 and 380 μM LV for optimal 5-FU potentiation. The more sensitive a cell line was to 5-FU alone, the more likely the cytotoxic effects were to be enhanced by LV.

We examined 5-FU/LV potentiation in five human cancer cell lines using short (1 h) 5-FU exposures with 20 μM LV added simultaneously with the 5-FU and left in the medium following removal of the fluoropyrimidine, in an attempt to mimic the more commonly used 5-FU bolus administration schedules. As illustrated in Figure 2, approximately 3-fold 5-FU IC_{50} decreases were observed with LV in the MCF-7 and MDA-231 breast

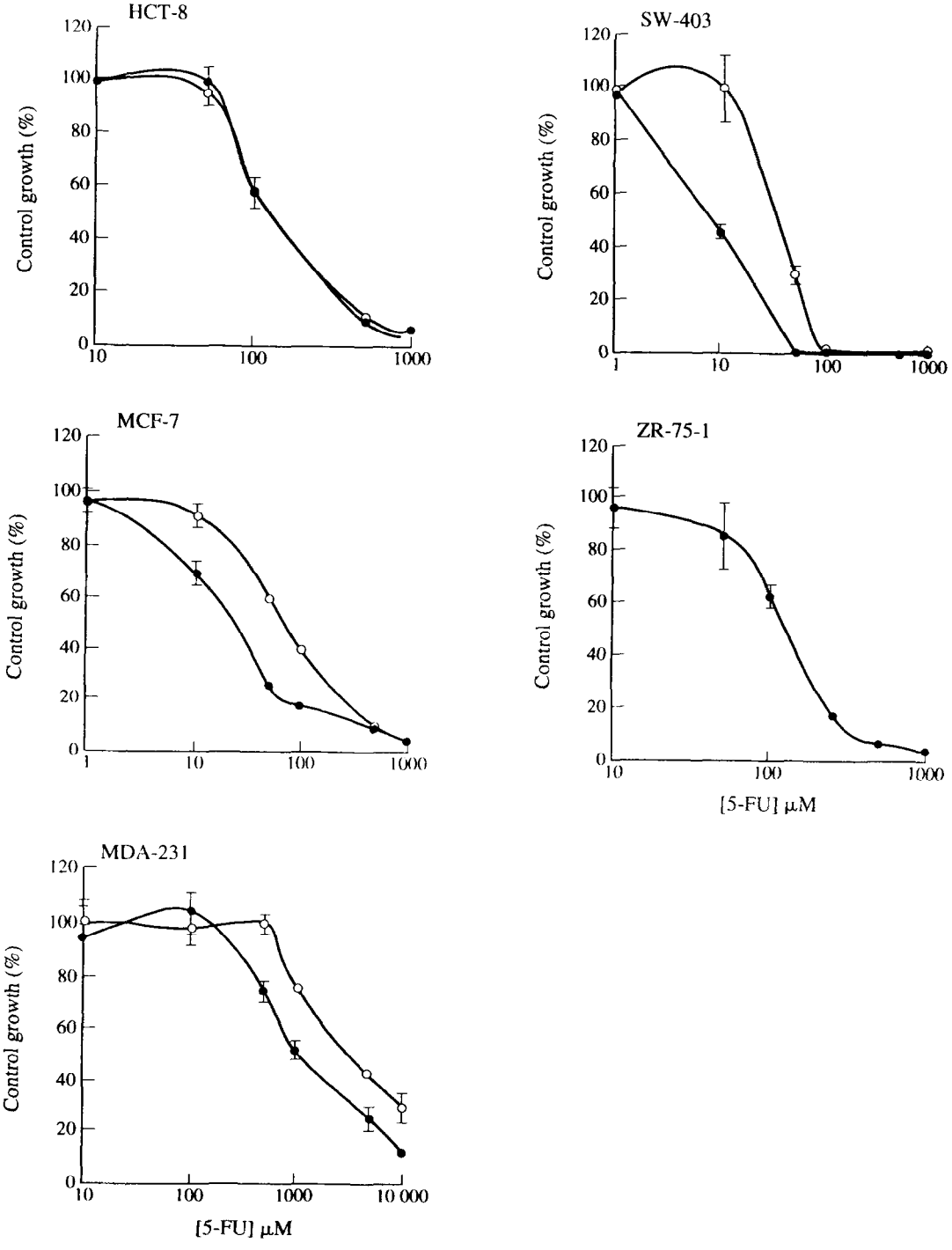

Figure 2. 5-Fluorouracil/leucovorin (5-FU/LV) growth curves in human cell lines. Human HCT-8 (obtained from Dr Abraham Fuks, McGill Cancer Centre, Montreal, Canada) and SW-403 (from the American Type Culture Collection, Rockville, Maryland, U.S.A.) colorectal cells and MCF-7, ZR-75-1 and MDA-231 breast cancer cell lines (from the National Cancer Institute, Bethesda, Maryland, U.S.A.) were exposed either to 5-FU for 1 h in the presence of 2 μM folic acid (open circles) or to 5-FU simultaneously with 20 μM LV which was also added to the media after 5-FU removal (solid circles). Cell growth was determined after three cell doubling times. Results represent the mean and standard deviation of three experiments.

cancer and the SW-403 colon cancer cell lines, with no effect on the breast ZR-75-1 and colon HCT-8 cells with this schedule. Interestingly, three of these lines were also examined by Beck and colleagues using prolonged 5-FU exposures [38]. Similar 5-FU potentiation by LV was obtained in the SW-403 cells, but 6- and 8-fold decreases in the 5-FU IC_{50} were seen in the MCF-7 and ZR-75-1 cells, underlying the importance of prolonged 5-FU exposures to elicit optimal LV potentiation in certain cell lines.

It is impossible to draw definitive conclusions as to the optimal 5-FU/LV dose scheduling based on the *in vitro* experimental data, but the bulk of the data suggests that, even though many cell lines derive little or no benefit from adding LV to 5-FU, prolonged exposures to 5-FU and LV are probably necessary to maximise LV potentiation.

In vivo

5-FU potentiation by LV has been studied in tumour-bearing mice treated with repeated daily and/or weekly 5-FU bolus injections with LV administered intraperitoneally or by intravenous bolus [35]. Modest delays in tumour growth have usually been achieved by these protocols [39, 40]. The suitability of this experimental model to study 5-FU/LV potentiation is restricted, however, by the high plasma thymidine levels in mice, and consequent tumour salvage through the thymidine kinase pathway [40]. Nevertheless, some information relative to LV dose scheduling has been obtained with this model. It was shown that LV can increase methyleneH_4folate polyglutamate levels in tumour-bearing mice [41] with prolonged (24 h) infusions being superior to shorter (4 h) LV infusions, and higher LV doses more effective than lower doses in potentiating 5-FU in certain tumours [26, 40].

CLINICAL TRIALS

Two 5-FU/LV administation schedules have been extensively evaluated clinically: a weekly and a 5-day intravenous bolus schedule. Both regimens were shown to yield superior response rates compared to 5-FU alone in randomised trials [42, 43]. The results from 10 randomised trials were subjected to a meta-analysis, confirming a highly significant benefit of 5-FU/LV therapy over single-agent 5-FU in terms of tumour response rate (23 versus 11%) without discernable improvement in overall survival [3]. Both drugs were administered by bolus injection in all these trials, except for one that used a continuous high dose (500 mg/m^2) 5-day LV infusion along with 5-day bolus 5-FU injections [44]. LV doses have been compared in three randomised trials. High (500 mg/m^2) and low (25 mg/m^2) LV doses were first compared in the weekly bolus schedule with the 500 mg/m^2 dose resulting in a higher objective regression rate [42]. Tissue biopsy studies carried out in patients receiving this administration schedule confirmed that high-dose LV increased thymidylate synthase inhibition compared to results obtained in biopsies from patients treated with 5-FU alone [45]. Interestingly, patients treated with the lower LV dose (25 mg/m^2) had less thymidylate synthase inhibition compared with the patients receiving the higher dose [46]. Both high (200 mg/m^2)- and low (20 mg/m^2)-dose LV were also compared in the 5-day bolus schedule with bolus 5-FU, and showed similar activity [47]. Similarly, the high-dose weekly schedule and the low-dose 5-day bolus schedules were compared in a recent randomised trial, and showed no significant differences in therapeutic efficacy with respect to objective tumour response, survival and palliative effects [48].

The above studies have established, with reasonable certainty, that 5-day bolus 5-FU and low-dose LV injections yield therapeutic benefits equivalent to that obtained with intravenous bolus schedules using higher doses of LV. It remains to be determined, however, if this is the optimal administration schedule. The *in vitro* studies summarised above would suggest that administrtion schedules incorporating prolonged (≥ 24 h) LV infusions producing micromolar (6S)-5-formylH_4folate plasma concentrations simultaneously with 5-FU infusions could possibly improve the combinations' therapeutic efficacy. Two clinical trials have examined weekly 24-h 5-FU and LV infusions. In one trial, (5-FU 2600 mg/m^2; LV 500 mg/m^2), 10 of 22 patients responded [49], while in the other (5-FU 2300 mg/m^2; LV 50 mg/m^2), 11 of 25 had partial responses [50]. One recent study has been investigating 5-FU (200 mg/day) and LV (10 mg/day) as a continuous 21-day infusion with 7 days of therapy, and reported an early 36% response rate [51]. Interestingly, there is a trend favouring survival for the 5-FU infusion arms in the recent SWOG screening trial of seven different 5-FU-based treatment schedules in patients with metastatic colorectal cancer [52]. There were no treatment arms with simultaneous infusions of both 5-FU and LV in this trial.

CONCLUSION

Clinical studies have established with reasonable certainty that 5-day bolus 5-FU and low-dose LV injections yield therapeutic benefits equivalent to that obtained with intravenous bolus schedules using higher doses of LV. The optimal administration schedules of 5-FU/LV-based chemotherapy treatment regimens still remain to be determined. The *in vitro* experimental data indicate that LV dose and length of administration and 5-FU length of exposure are all important parameters in optimising 5-FU/LV therapy and that no fixed dose-schedule can determine optimal potentiation in all cell lines. It would appear that prolonged cellular exposures (≥ 24 h) to relatively low (μM) LV concentrations simultaneously with prolonged 5-FU administration is a strategy worthy of further clinical investigation.

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