Critical factors for optimizing the 5-fluorouracil-folinic acid association in cancer chemotherapy

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Summary

Background: The 5-fluorouracil (FU)-folinic acid (FA) association has demonstrated clinical efficacy in colorectal cancer, both in adjuvant and metastatic situations. However, there is no clear consensus about the optimal FU-FA schedule and dose. In addition, it would be of interest to identify FU-FA-responsive tumors.

Design: Our purpose was to review preclinical and clinical data dealing with prediction of FU-FA sensitivity and optimization of FU-FA schedules.

Results: Preclinical studies have highlighted the importance of thymidylate synthase (TS), the cellular target of the FU-FA mechanism of action, for predicting FU sensitivity. It appears that the more sensitive cell lines express the lowest TS activity. Interestingly, the cell lines sensitive to FA supplementation are those more sensitive to FU. The role of TS in FU-FA responsiveness has been clearly demonstrated in patients with colorectal and gastric cancers. Preliminary in vitro and clinical data have shown that the folylpolyglutamate synthase (FPGS), the enzyme responsible for folate polyglutamation, is another promising tool for identifying FU-FA-responsive tumors.

So far, results of clinical trials do not form a clear consensus regarding the need to administer high FA doses for improving FU-FA treatment. Experimental studies on human cancer cell lines have demonstrated the wide variability among cell lines, ranging from 0.05 to 200 μM, of FA concentrations required for maximal FU potentiation. In addition, pharmacokinetic studies have reported a significant variability of active folates in plasma after administration of standard-dose FA. Altogether, these observations favour high-dose FA administration to achieve high folate concentrations in plasma and thus to counteract the variability of the FA concentrations required. With respect to the choice of FU-FA schedule, it appears from experimental data that increasing the duration of exposure to FA enhances FU-FA cytotoxicity, probably through an increased formation of reduced folate polyglutamate forms. Considering the S-phase specificity of FU cytotoxicity as well as its rapid elimination from plasma, a schedule of prolonged exposure to both FU and FA should be considered preferable.

Conclusions: Results of the new FU-FA administration schedules such as the one consisting of a 2-hour FA administration followed by a combination of FU bolus and FU infusion, or the chronomodulated FU-FA infusion, open up promising approaches for improving the therapeutic index of FU-FA chemotherapy. Finally, future clinical studies should investigate tumoral parameters pharmacologically linked to FU-FA sensitivity such as pre-treatment TS and FPGS activities. Such tumoral investigations along with FU and FA pharmacokinetic investigations should provide a better understanding of inter-patient variability in response to FU-FA therapy and an optimal management of this chemotherapy regimen.

Key words: 5-fluorouracil, folinic acid, pharmacomodulation

Introduction

Despite being one of the oldest anticancer drugs, 5-fluorouracil (FU) is increasingly being used in cancer chemotherapy and occupies a major place not only in the treatment of advanced colon cancer but also head and neck cancer [1] and breast cancer [2]. One explanation for this phenomenon is the wide range of possibilities of FU pharmaco-modulation which markedly increase the antitumor efficacy of this antimetabolite [3]. Of the different approaches to FU modulation, the association with folinic acid (FA) is a major one [3].

FU is activated through different pathways leading to at least 3 cytotoxic compounds: FdUMP, which inhibits thymidylate synthase (TS) and subsequent DNA synthesis, FUTP which is directly incorporated into RNA, and FdUTP for which incorporation into DNA has been advocated [4]. The relative importance of these pathways seems to depend on the dose administered and the schedule. Yet, experimental studies have demonstrated that short-term exposure to high concentrations of FU kills the cells preferentially by an RNA effect whereas prolonged exposure to low concentrations is cytotoxic mainly via TS inhibition [5]. Enhancement of FU cytotoxicity by FA is based on the optimal inhibition of TS resulting from an increase in the intracellular pool of 5–10 methylene-tetrahydrofolate (CH₂FH₄) which, in turn, stabilises the inactive complex formed between TS and fluorodeoxyuridine monophosphate (FdUMP) [6]. Synthesis of FA
results in a racemic mixture (dl FA, Leucovorin®) of the 2 diastereoisomers l FA (S,S FA) and d FA (R,S FA). It has been demonstrated that biological activity of the racemic mixture dl FA is supported by the natural l FA isomer (Elvorine®), with d FA having little, if any, biological activity [7]. Numerous studies have established the beneficial effects of FU-FA therapy in advanced-cancer patients for objective response [8-15] and survival [16]. Noteworthy is the fact that recent randomized trials comparing FU-FA versus no treatment as adjuvant therapy following surgery in colorectal cancer patients have demonstrated an advantage for FU-FA in terms of disease-free survival [17-19] and overall survival [17,18].

The clinical use of FU-FA combined chemotherapy is still hampered by unresolved issues such as the choice of optimal schedule and optimal FA dose as well as the identification of FU-FA-responsive tumors. The main objective of the present review is to analyze the different factors which must be taken into account when seeking to answer these questions and make proposals for optimizing the clinical use of FU modulation by FA.

**Perspectives for identifying FU-FA-responsive tumors**

Thymidylate synthase (TS) is considered the main target of FU action when given by continuous intravenous infusion [5, 20]. Both FU and FA can be considered as prodrugs, given their interaction with TS, since TS inhibition is linked to the formation of an inactive ternary complex between TS, FdUMP coming from FU and CH₂FH₄, of which FA is one of the precursors. Since both FU and FA anabolites interact on TS, an elevation of TS could be a cause of resistance to FU treatment as well as to FU-FA combination therapy. In preclinical models, overexpression of TS is not universally recognised as a determinant factor for FU resistance [5, 21, 22]. Beck et al. [23, 24] recently provided experimental data on human tumor cell lines exhibiting inherent sensitivity to FU. They showed that the greater the FU efficacy, the lower the basal TS activity [23] and, interestingly, they noted that the sub-group of cell lines responding to FA supplementation was the one that was intrinsically more sensitive to FU [24]. This observation suggests that FU-sensitive tumors are the best candidates for FA supplementation and adds authority to the proposal that tumoral TS activity be considered a predictor for response to FU-FA chemotherapy. In fact, a number of clinical investigations performed in breast [2] and digestive-tract cancer patients [25-29] have suggested or demonstrated that overproduction of TS was related to FU-FA resistance. In contrast, no relationship was demonstrated between FU responsiveness and overexpression of tumoral TS in head and neck cancer patients [30].

Beyond the critical role of pre-treatment TS activity or expression, some studies have highlighted the importance of the FU-induced increase of TS and of the duration of TS inhibition following FU ± FA chemotherapy in colon cancer [26, 31-33]. Van der Wilt et al. [31] have shown that FU therapy induced an elevation of total TS levels in vitro. Interestingly, the combination of FA with FU reduced this increase in TS [31]. In colon cancer patients, Peters et al. [32] have suggested that maintenance of TS inhibition was a predictor of treatment response and proposed threshold values for free binding sites and residual TS catalytic activity under FU ± FA treatment for predicting response. Moreover, they demonstrated that FU-FA administration resulted in TS inhibition significantly enhanced over that of FU treatment alone [32].

The TS protein is constituted of two identical subunits, each comprising a nucleotide binding site and at least one folate binding site [34]. The described mutations in the TS protein can lead to a reduced affinity for the nucleotide [35] or the folate [36] or both [37]. The occurrence of these mutations may render more complex the prediction of FU-FA sensitivity based on TS activity or TS expression measurement. Hogan and Berger [38] have recently investigated a colon tumor cell line isolated from a patient not previously exposed to chemotherapy. This cell line was intrinsically highly resistant to fluorodeoxyuridine (FdUrd); however, FdUrd sensitivity was increased 10-fold by FA. Analysis of TS cDNA revealed a point mutation creating a restriction fragment-length polymorphism in DNA which could represent a potential marker for identifying tumors requiring FA modulation. Confirmation of such research in TS mutation should be of interest during clinical studies.

Since the intracellular concentration of CH₂FH₄ is a critical factor for optimal TS inhibition, the importance of pre-treatment tumoral CH₂FH₄ concentrations for identifying tumors requiring FA supplementation might be questioned. Surprisingly, data on CH₂FH₄ concentrations in tumors from pre-treated cancer patients are sparse [39-42]. Among the few studies available, Kühl et al. [41] investigated 17 patients with colorectal carcinoma. In 11 of 17 patients, CH₂FH₄ concentrations were below the detection limit of the CH₂FH₄ assay and for the remaining 6 patients the mean ± SD was 11 ± 25 pmol/mg protein [41]. This wide variability was also stressed by Dohden et al. [40] who reported a 78% coefficient of variation in tumoral CH₂FH₄ concentration in a group of 35 patients with digestive tract cancer. We recently investigated CH₂FH₄ concentrations in tumors from pre-treated cancer patients and confirmed this wide inter-patient variability, with CH₂FH₄ concentrations ranging between 0.3 and 8.2 pmol/mg prot [42]. We also investigated the link between intracellular CH₂FH₄ concentrations and FU cytotoxicity in the presence of FA supplementation on 9 human cancer cell lines [42]. Intracellular CH₂FH₄ concentrations allowing 90% of maximal FU potentiation were very close together, ranging from 8 to 15 pmol/mg prot...
between cell lines, and were globally higher than the tumoral CH₂FH₄ reported in the clinical setting using the same assay for CH₂FH₄ measurement [42].

The perception of tumor-to-tumor variability in CH₂FH₄ levels is interesting. It has been shown by several investigators that reduced folates inside the cells are present as polyglutamated forms with 2 to 10 glutamyl residues [43, 44]. The polyglylutamate synthetase (FPGS) is the enzyme responsible for folate polyglutamylallation. Polyglutamated CH₂FH₄ exhibits an increased affinity for TS as compared to the monoglutamate form [45, 46]. Polyglutamylation also increases the intracellular retention of reduced folates [47] which probably, to a great extent, accounts for the tumor-to-tumor variability in CH₂FH₄ concentrations. The critical role of polyglutamylation with respect to the biochemical modulation of fluoropyrimidines by FA was recently emphasized by Romanini et al. [48]. These authors showed that FA potentiated FU efficacy in the parental CCRF-CEM cell line, but not in the CCRF-CEM/P cell line with impaired ability to form polyglutamates. Interestingly, although levels of CH₂FH₄ were similar in both cell lines after FA administration, polyglutamated forms were markedly decreased in the CCRF-CEM/P cell line [48]. Houghton et al. [49] made a similar observation on a human colon adenocarcinoma transplanted in mice. These authors observed that tumors non-responsive to the FU-FA combination were those which were unable to expand their intracellular pool of polyglutamates. In addition, Wang et al. [50] have reported decreased activity of FPGS in a colon cancer cell line becoming resistant to FU treatment. We recently demonstrated on a panel of 14 cancer cell lines that FU sensitivity with or without FA supplementation was significantly related to the basal FPGS activity; the higher the FPGS activity, the greater the FU sensitivity (data submitted for publication). Also, preliminary data on FPGS activity measured in 35 biopsies of liver metastases obtained in colorectal cancer patients before FU-FA chemotherapy have shown that the response rate was significantly higher in metastases exhibiting the greater FPGS activity in comparison with metastases with impaired basal FPGS activity [29]. Taken together, the above data on intratumoral concentrations of reduced folates and their polyglutamylation have potential implications for optimizing the clinical use of FA in association with FU. Future studies should be encouraged in specimens of colorectal carcinoma or other tumor localizations responding to the FU-FA combination, such as head and neck cancers, to further investigate whether FPGS activity is a reliable predictor for the efficacy of FU-FA chemotherapy in the clinical setting.

Rationale for optimizing the FU-FA schedule

The question of the optimal FA dose is justified by the significant level of toxicity of FU-FA chemotherapy which is linked to both the FU and FA doses, and to the administration schedule, as demonstrated in preclinical studies as well as in animals [49] and the clinical setting [51]. So far, results of clinical trials form no clear consensus regarding the need to administer high FA doses in order to optimise FU-FA treatment. In metastatic disease, the Gastrointestinal Tumor Study Group [14] reported a significant improvement in response rate in patients receiving 500 mg/m² Leucovorin* over that of patients receiving 25 mg/m² Leucovorin*, using the same schedule and FU dosage (2-hour infusion of Leucovorin* + 600 mg/m² FU i.v. bolus). In contrast, a comparative trial from the North Central Cancer Treatment Group [16] in which both FU (370 mg/m²/d) and Leucovorin* were administered by rapid i.v. injection for 5 consecutive days has shown the same response rate with a lower FA dose (20 mg/m²/d versus 200 mg/m²/d) [16]. More recently, a comparison between low- and high-dose Leucovorin* published by the same group in advanced colorectal cancer patients [52] demonstrated no marked differences between low- and high-dose Leucovorin* regimens. In this study, the low-dose Leucovorin* regimen was significantly associated with a higher rate of hospitalization for toxicity (consisting of leukopenia, diarrhea and oral mucositis), but since the FU dose was greater in the low-dose Leucovorin* arm, no clear conclusion could be drawn from this trial [52]. A comparison of low FA dose versus high FA dose for FU-FA therapy given adjuvantly has not yet been published. However, in the 2 positive trials which demonstrated a benefit for FU-FA over no treatment in terms of overall survival, high FA doses were administered [17, 18]. In the study of O'Connell et al. [19] comparing a low FA dose plus FU therapy given adjuvantly versus no treatment, a benefit for FU-FA was demonstrated for disease-free survival only.

Experimental studies on human cancer cell lines [24, 53, 54] suggest that the optimal concentrations of 1 FA necessary for maximal FU potentiation are quite different among cell lines. This question of the optimal FU concentration was addressed in a study by Beck and colleagues [24] on a large panel of cancer cell lines. On 17 human cancer cell lines representative of the localizations responding to FU-FA treatment, these authors reported tremendous variability in the optimal FU concentration required for maximal FU modulation [24]. For the 11 cell lines responding to FA supplementation, a 4000-fold range of 0.05 to 200 μm in optimal FU concentration was observed. In this study [24], the FU concentration necessary for an optimal FU cytoxicity enhancement was in the range of 0.5–5 μm in 5 cell lines, in accord with data from Moran [53] and Zhang [54] obtained with 3 cell lines. This marked variability in FU concentrations contrasts with the low variability of intracellular CH₂FH₄ concentrations required for FU modulation, as pointed out above [42]. Stereosepecific pharmacokinetic studies of FA-treated patients showed that after a single oral or i.v. administration, FA is rapidly cleared from plasma with a terminal half-life of around 30–60 minutes whereas its
active metabolite 1 5-methyltetrahydrofolate (5MTHF) exhibits a greater half-life of around 5–10 hours [55, 56]. When administered intravenously, FA pharmacokinetics have been demonstrated to be linear with a dose of between 10 and 500 mg/m² [56]. Following a single i.v. administration of 500 mg/m² Leucovorin®, maximal plasma concentration of FA and 5MTHF are around 50 and 6 µm, respectively [56]. The same dose given as a continuous infusion (500 mg/m²/d) led to mean steady state concentrations of 3 µm for FA and 6 µm for 5MTHF [55]. In addition, pharmacokinetic studies have shown a wide inter-patient variability for both FA and 5MTHF plasma concentrations after administration of standard FA doses [55, 57]. Altogether, these observations are in favour of high-dose FA administration in the clinical setting to achieve high folate concentrations in plasma and thus to counteract the wide variability of FA concentrations required for optimal FU efficacy. In agreement with this view, a recent study performed in head and neck cancer patients receiving cisplatin-FU-1 FA therapy has shown that non-responding patients exhibited significantly lower FA + 5MTHF plasma concentrations than responding patients [57]. In addition, numerous studies have shown the wide variability in FU concentrations measured in plasma following administration of a standard FU dose [58, 59]. Moreover, pharmacokinetic-pharmacodynamic studies performed in colorectal cancer patients [60] and head and neck cancer patients receiving FU-FA treatment have demonstrated that responding patients exhibited significantly higher FU plasma concentrations than non-responding patients. In head and neck cancer patients receiving cisplatin-FU treatment, it was also demonstrated that the FU systemic exposure was an independent prognostic factor for overall survival [61].

There is no clear consensus about either the optimal FA dose nor administration schedule for either FA or FU, which are currently administered by either daily bolus injection [16], or short or continuous infusion [57, 62]. Experimental investigations on colorectal and bladder cancer cell lines exposed to various FU-FA schedules showed that increasing the duration of exposure to 10 µm FA from 1 to 72 hours continuously enhanced the cytotoxicity of 72-hour exposure to FU [53]. An explanation for this observation is provided by Boarman and Allegra’s experimental study [47] which stressed the critical importance of FU exposure duration in the formation of polyglutamates of reduced folates (Glu3-Glu5). Moreover, these authors emphasized that the intracellular half-life of the polyglutamate forms was inversely related to the length of the polyglutamate tail [47]. The advantage of a continuous as opposed to a shorter FA administration (24 hours versus 4 hours) was also suggested in an experimental study by Houghton et al. [63] showing that the intratumoral levels of CH₃FH₄ and tetrahydrofolate declined very rapidly after termination of infusion, as did reduced folates in plasma. To our knowledge, only one clinical study comparing two FU-FA administration schedules has reported CH₃FH₄ intratumoral concentrations [39]. Surprisingly, in this study performed in a limited number of advanced colorectal cancer patients, tumoral CH₃FH₄ concentrations measured during a 5-day continuous infusion of FA (2 patients) were lower than those observed after a 2-hour infusion schedule (4 patients). However, the analysis of CH₃FH₄ polyglutamated forms was not reported in this study [39].

In theory, considering the S-phase specificity of FU cytotoxicity and the short elimination half-life of FU in plasma, the optimal schedule would appear to increase the duration of FU exposure. In fact, the superiority of continuous infusion over shorter administration schedules has been demonstrated in metastatic colorectal cancer [64] and head and neck cancer [65]. In light of all these factors, prolonged exposure to both FU and FA should be considered the optimal schedule. A still partially unresolved issue concerns the impact of prolonged FU-FA exposure on normal tissues sensitive to FU-related toxicity. A recent clinical study by De Gramont et al. [62] comparing a low-dose FA (20 mg/m²) plus FU given by i.v. bolus for 5 days every 4 weeks (arm A) versus a high-dose FA (200 mg/m²) given over a 2-hour infusion plus FU given by i.v. bolus followed by a 22-hour infusion, for 2 days every 2 weeks (arm B) demonstrated, as expected, a significantly greater response rate in arm B. Arm B was also associated with a significant improvement in progression-free survival and, interestingly, the incidence of grades 3–4 toxicities was significantly lower in this arm [62]. The chronomodulation of FU-FA infusion is also a promising approach. In 2 randomized trials comparing a flat infusion versus a chronomodulated infusion (FU-FA given between 10 p.m. and 10 a.m. with a peak at 4 a.m.), Levi et al. [66] demonstrated that chronomodulation of FU-FA associated with oxaliplatin significantly improved the objective response rate in advanced colorectal cancer patients while significantly decreasing the incidence of toxicity. Further clinical trials comparing different FU-FA schedules should evaluate in detail the antitumor efficacy/toxicity ratio in order to improve FU-FA chemotherapy.

Conclusions

The FU-FA combination has demonstrated clinical efficacy both in adjuvant [17–19] and metastatic [8–16] situations. Although there is no clear consensus as to the optimal FA dose and schedule, FU-FA combined chemotherapy is considered to be a reference protocol in colorectal cancer patients. The difficulty in answering the question of optimal dose and schedule stems from the fact that the dose and schedule of both FA and FU closely affect one another. For instance, administration of a low FA dose allows a higher FU dose to be administered [52]. Thus, the modification of all of
these parameters encountered in clinical trials makes it difficult to draw clear conclusions. As regards the FA dose, experimental data have demonstrated the tremendous variability in efficient FA concentrations for reaching optimal FU potentiation [24, 53, 54]. This observation suggests that high FA doses should be used in order to overcome this variability, as far as possible. A second factor of variability concerning the choice of FA dose derives from the wide inter-patient variability of FA pharmacokinetics, suggesting the potential importance of FA pharmacokinetic follow-up with dose adjustment.

The end point of clinical trials comparing different FU-FA doses and schedules must encompass not only the therapeutic efficacy of the tested regimens but also toxicity and quality of life. Results of the new FU-FA administration schedules such as the one suggested by De Gramont [62] or chronomodulated FU-FA infusion [66] open up promising approaches for improving the therapeutic index of FU-FA chemotherapy. Regarding the choice of FU-FA schedule and dose for future investigations, relevant pharmacologic data come from studies performed in colorectal cancer patients. At present, one of the reference protocols in adjuvant therapy of colorectal cancer is the daily schedule initially proposed by Machover et al. [17, 18] which consists of high-dose FA followed by FU, both given as short i.v. for 5 days every 4 weeks. From a recent randomized trial, the schedule proposed by De Gramont et al. [62] consisting of a 2-hour FA administration followed by a combination of FU bolus and FU infusion (for 2 days every 2 weeks) appears to be better than the Machover schedule in the treatment of metastatic disease. Interestingly, the FU dose-intensity in this latter schedule is 2-fold higher than that of the Machover schedule. It would thus be of interest to compare these two FU-FA schedules as adjuvant treatment of colorectal cancer patients. Such a multicentric trial is being planned in France.

The second point which merits consideration in future FU-FA strategies is the search for predictors of FU-FA sensitivity. Clinical data have shown that colorectal and gastric tumors likely to respond to FU-FA therapy are those exhibiting lower TS levels. Future clinical studies should investigate tumoral pharmacokinetic investigations, such as pre-treatment TS but also FPGS activity. Such tumoral investigations along with FU and FA pharmacokinetic studies should provide a better understanding of inter-patient variability in response to therapy and of the optimal management of the FU-FA association.

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