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Phase II Trial of 5-Fluorouracil and the Natural *l* Isomer of Folinic Acid in the Treatment of Advanced Colorectal Carcinoma

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Between February 1991 and July 1992, 79 previously untreated patients with metastatic colorectal carcinoma were enrolled in a phase II study of combined 5-fluorouracil (5-FU) and *l*-folinic acid (FA). 5-FU 370 mg/m²/day was administered for 5 consecutive days as an intravenous (i.v.) bolus injection preceded by *l*-FA 100 mg/m²/day with the same administration modality. Treatment was given every 4 weeks until progression. 79 patients were evaluable for toxicity and 64 for response. 2 patients (3%) achieved a complete remission and 8 (12.5%) a partial remission, 33 (52%) had stable disease and 21 patients (33%) had progressive disease. Median duration of remission was 32.5 weeks and median survival for all evaluable patients was 64.5 weeks. Substantial to severe side-effects occurred in 39% of patients. Dose-limiting toxicity (grade 3–4) was mainly diarrhoea (18%) and mucositis (15%). Nausea/vomiting, cutaneous toxicity, leucopenia, alopecia and conjunctivitis of grade 3–4 occurred respectively in 6, 4, 2.5, 1 and 1% of cases. Toxicity appeared to be substantially similar to that characteristic of combined 5-FU and the chiral mixture of *d,l*-FA. Efficacy was within the range of that observed with the 5-FU/*d,l*-FA combination, although at the lower level.
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INTRODUCTION

RELEVANT CLINICAL results have recently been obtained in the treatment of patients with advanced gastrointestinal carcinomas by selectively potentiating the antitumour activity of 5-fluorouracil (5-FU) with modulating drugs (e.g. methotrexate, phosphonacetyl-L-aspartic acid, folinic acid) which interfere with its metabolic pathways [1].

In particular, the ability of folinic acid (FA) to enhance the clinical efficacy of 5-FU has been demonstrated in patients with colorectal adenocarcinoma; the objective response rate after treatment with 5-FU and FA has been reported to be as high as 13% in patients previously exposed to 5-FU alone and 30% in previously untreated patients [1, 2]. The majority of comparative trials have also demonstrated a significant advantage of the combination of 5-FU and FA over 5-FU alone in terms of objective response [3–10], and some studies have also shown increased survival [4, 5]. However, a minority of these studies contradict the general trend, not showing significant differences in response [11, 12]. A meta-analysis, including all published phase III studies, suggests that 5-FU and FA may offer a definite advantage over 5-FU alone in the treatment of advanced colorectal cancer [13].

In several human tumour cell lines [1, 2] and in tumour explants from patients [14–16], the extent and duration of the inhibition of thymidylate synthase by fluorodeoxyuridylate, the main active metabolite of 5-FU, constitute important determinants of sensitivity to the fluoropyrimidine.

Preclinical and clinical studies have shown that increased concentrations of the cofactor 5,10-methylenetetrahydrofolate, which are generated after administration of FA, enhance forma-

tion and stability of the ternary complex among fluorodeoxyuridylate, thymidylate synthase and this folate cofactor, leading to potentiation of the DNA-mediated effects of 5-FU [1, 2, 16].

Commercially available FA has consisted, up to now, of an equimolar mixture of two stereoisomers differing in chirality at the asymmetric centre represented by carbon 6 of the pteridine ring. Only the natural *l* isomer is biologically active, while the unnatural *d* isomer is not metabolised, and reaches high concentrations in the plasma (in the range of 10–100 μM at peak), greatly exceeding contemporaneous levels of *l*-FA over a long period of time, following intravenous (i.v.) administration of high doses (≥200 mg/m^2) of the *d,l* mixture [17–20]. The half-life of *d*-FA is, in fact, about 5–10-fold longer than that of the *l* form (ranging, respectively, from 6.4 to 11 h and from 0.75 to 2 h).

Under these circumstances, it is theoretically possible that the *d* isomer interferes with the transport and intracellular metabolism of the *l* isomer, and with its induced enhancement of the ternary complex formation and stability, leading to impairment in the effectiveness of 5-FU chemotherapy.

Although only *l*-FA is converted into active folate cofactors, the *d*-isomer is not inert. It has been demonstrated that it competes with the natural *l* isomer, although poorly (k_i in the range of 30–50 μM), for intracellular transport [21, 22]. After cell uptake it can be polyglutamylated by folylpolyglutamate synthetase since both isomers are active substrates for this enzyme [23], which has a critical role in the folate-induced modulation of fluoropyrimidine cytotoxicity [24, 25]. Although it has been suggested that *d*-FA cannot be converted to other folate forms via methenyltetrahydrofolate synthetase, given its stereospecificity for *l*-FA [26], the unnatural *d*-isomer may interact directly with thymidylate synthase. At high concentrations (in the mM range) *d*-FA can, in fact, inhibit thymidylate synthase activity [27]. It has also been shown that at lower concentrations (in the μM range) *d*-FA is unable to facilitate the binding of fluorodeoxyuridylate to thymidylate synthase [28]. Thus, while it is unlikely that the unnatural isomer of FA serves as an effector for binding of fluorodeoxyuridylate to thymidylate synthase in replacement of the natural cosubstrate for the enzyme, 5,10-methylenetetrahydrofolate, it is conceivable that it plays a negative interfering role, inhibiting the formation of a stable 5-FU-mediated ternary complex, especially at the high concentrations accumulated following administration of high *d,l*-FA doses.

A substantial lack of interference of *d*-FA with the potentiation of fluoropyrimidine cytotoxicity mediated by the natural *l*-FA isomer has been demonstrated in human tumour cell lines *in vitro* [23, 29, 30], as well as in murine tumours *in vivo* [31]. However, the described and potential cellular effects of the unnatural *d*-isomer of FA support the hypothesis of its detrimental effect on the modulation of fluoropyrimidines by *l*-FA. These interactions can be avoided by use of the pure pharmaceutical preparation of *l*-FA currently available in Italy.

The present study was undertaken to evaluate the toxicity and efficacy of combined 5-FU and *l*-FA at high stereochemical purity in a 5-day monthly therapy of previously untreated patients with advanced colorectal carcinoma. The dose of *l*-FA, 100 mg/m^2/day, was half the standard dose of the equimolar *d,l* isomer mixture, previously studied in combination with 5-FU [17].

Following our preliminary report [32], we present here the final analysis of our study results.

PATIENTS AND METHODS

Patients

Admission criteria were diagnosis of advanced colorectal carcinoma (evidence of metastatic disease or unresectable recurrence); age ≤75 years; performance status ≤2 (WHO); life expectancy >3 months; measurable lesions; total bilirubin <5 mg/100 ml and transaminase levels ≤5 × normal value; adequate bone marrow function (leucocytes ≥4000/mm^3 and platelets ≥100 000/mm^3). Patients were registered at the single participating institutions prior to treatment and notification of registration was given to the coordinating centre within 1 week.

Patients were permitted to have had adjuvant chemotherapy if the treatment had ended at least 12 months before admission to the study, but they were required not to have had previous chemotherapy for metastasis; patients with previous radiotherapy were eligible only if the treatment was performed at least 4 weeks before the beginning of chemotherapy. During treatment, radiation therapy was allowed on unresponsive lesions to palliate pain.

Oral informed, independently witnessed consent was obtained according to national and institutional requirements.

Treatment

Patients were treated with a regimen that consisted of 5-FU (Roche, Italy) 370 mg/m^2 administered by i.v. bolus for 5 consecutive days immediately preceded by *l*-FA (Cyanamid Lederle, Italy) 100 mg/m^2 i.v. bolus. The cycle was repeated every 4 weeks, until progression.

The pharmaceutical *l*-FA preparation used contained 0.5% or less of the unnatural *d* stereoisomer. The prognostic relevance of base-line variables to therapeutic response was investigated by means of either the χ^2 test or one-way ANOVA.

Evaluation

Before the beginning of treatment, a complete history, full physical examination, laboratory tests [white blood cells (WBC), red blood cells (RBC), platelets (PLT), haematocrit, haemoglobin, bilirubin, transaminases, gammaglutamic acid, alkaline phosphatase, carcinoembryonic antigen], chest X-ray, abdominal computed tomography (CT) scan and/or ultrasound were performed. Clinical evaluation was performed every two cycles, and the laboratory assessment and physical examination every cycle.

Criteria for response were those recommended by the WHO [33]. In particular, complete response (CR) was defined as disappearance of all lesions; partial response (PR) as ≥50% reduction in measurable tumour masses in the absence of new lesions; stable disease (SD) as <50% decrease or ≤25% increase of one or all measurable lesions; progressive disease (PD) as appearance of new lesions or >25% increase of pre-existing ones.

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The duration of CR was calculated from the time of its occurrence until progression or the last examination, while that of PR and SD was calculated from the beginning of chemotherapy.

Survival was calculated from the start of chemotherapy. Survival analysis was performed according to the Kaplan–Meyer method. Comparison of the survival curves were made using the log rank test.

Toxicity was evaluated according to WHO criteria [33]. In case of grade 1 leucopenia or thrombocytopenia, therapy was interrupted until normalisation. If the same parameters were evaluated as grade 2, treatment was interrupted until normalisation and then 75% of the 5-FU dose was administered. The dose was 50% in case of grade 3–4 myelosuppression. If grade 2–4 mucositis or diarrhoea occurred, treatment was interrupted until normalisation and then 50% of the 5-FU dose was administered: in case of good tolerance at this dose level, the following cycles were performed with an increase of 100 mg/m^2/day every course until the initial dose was reached. In contrast, if tolerance was bad, the 5-FU dose was reduced to 25% of the initial one. For all other grade 3–4 toxicity types (except alopecia), 50% of the 5-FU dose was administered.

RESULTS

Patients

79 patients entered the study between February 1991 and July 1992 and all were eligible. Patients were in good general condition with a median performance status of 0, and a median age of 62 years. 50 patients were male and 29 were female. Disease sites were primarily hepatic (51 cases), pulmonary (28 cases) and lymphonodal (11 cases), varying from one to four per patient (median 1). The median number of cycles administered per patient was four with a range of one to 15 (Table 1). All patients were previously untreated except one who had received postoperative adjuvant 5-FU with FA more than 12 months before.

Table 1. Patients' characteristics

No. patients	79
Male/female	50/29
Age (years)	
Median	62
Range	39–75
Performance status (WHO)	
0	45
1	29
2	5
No. of cycles	
Median	4
Range	1–15
No. of disease sites	
Median	1
Range	1–4
Primary site of disease	
Colon	58
Rectum	21
Disease sites	
Liver	51
Lung	28
Lymph nodes	11
Peritoneum	7
Pelvis	6
Primary tumour/ local recurrence	4
Bone	3
Omentum	2
Others*	6

* 1 mediastinum, 1 pleura, 1 skin, 1 spleen, 1 psoas, 1 presacral site.

Response

64 patients were evaluable for response. 15 patients were considered inevaluable for response because of early death due to tumour or early disease progression (3 cases), early withdrawal for toxicity (2 cases) or patient refusal (2 cases), or protocol violations (6 cases). 2 patients were lost to follow-up within the first 2 months of the study. Treatment with 5-FU and *l*-FA caused a CR in 2 (3%) patients. 8 patients had a PR (12.5%), and 33 (52%) SD, while PD after the first two treatment cycles occurred in the remaining 21 patients (33%). The durations of the two CR were 55 and 75+ weeks; those of PR ranged from 13 to 44 weeks (median 27.5 weeks) (Table 2). The two CR observed were achieved after 3 and 5 months of therapy, respectively. In 7 of 8 partial responders, remission was obtained after 2 months and in 1 patient after 6 months. The overall response rate was 15.5% (95% confidence interval, 7–24%) and the overall median duration of response was 32.5 weeks.

Of the initial clinical parameters, only the number of sites of metastases proved to be of borderline significance on response rates. In fact, 8 of 39 evaluable patients with one site of metastasis responded to therapy, but only 2 of 25 patients with two or more sites responded ($P = 0.16$). All other variables tested such as performance status, age, sex and primary site of tumour had no influence on remission rate. However, none of the 4 patients with WHO 2 performance status achieved a response, whereas 10 of 60 patients with WHO 0–1 did achieve remission ($P = 0.49$).

No specific metastatic site appeared to respond better than any other; the 10 responses occurred in patients with liver

Table 2. Therapeutic efficacy in 64 evaluable patients

Response	No. of patients	(%)
Complete	2	(3)
Partial	8	(12.5)
Stable disease	33	(52)
Progressive disease	21	(33)
Median duration of complete response (weeks)		65
Range (weeks)		(55, 75+)
Median duration of partial response (weeks)		27.5
Range (weeks)		(13–44)

disease only (5 patients), lymphonodal disease only (1 patient), lung disease only (1 patient), pelvic disease only (1 patient), liver and lung (1 patient), and lymphonodal and primary tumour (1 patient).

Survival

58 patients have died, 19 patients are living and 2 have been lost to follow-up (August 1993). Median survival for all patients evaluable for response (64) was 64.5 weeks (range 9–115+), for those with CR + PR was 94 weeks (range 20–111+), and for SD was 69.5 weeks (range 18–100+). The median survival for patients with PD was 43.5 weeks (range 9–115+). The differences between survival of patients with CR + PR or with SD and that of patients with PD were significant ($P < 0.01$).

Toxicity

All 79 patients were evaluable for toxicity. Toxicity was manifested primarily as gastrointestinal side-effects, with 73% of patients experiencing mucositis, 54% diarrhoea and 49% nausea and vomiting. Other side-effects such as leucopenia (16%), alopecia (16%), cutaneous toxicity (16%), conjunctivitis (13%), asthenia (11%), abdominal pain (10%), and infection (8%) were less frequent while possibly or probably related cardiac toxicity (5%), and thrombocytopenia (3%) were relatively rare (Table 3). 2 patients developed ageusia, one phlebitis, and one peripheral neurotoxicity, in all cases of mild to moderate degree (Table 3). Mucositis of WHO grades 3–4 was reached in 15% of patients, and diarrhoea of the same degree in 18% of patients. Substantial to severe nausea/vomiting, cutaneous toxicity and leucopenia occurred in 6, 4 and 2.5% of patients, respectively. These degrees were also reached in 1% of patients experiencing infection, cardiotoxicity, alopecia and conjunctivitis. Toxic effects caused a 25% reduction of the 5-FU dose in 43 cycles (12%) and a 50% dose reduction in 31 cycles (9%) of the 352 administered. A 25% 5-FU dose reduction occurred in 7 patients (9%) and a 50% dose reduction in 16 (20%). In 20 of these cases (87%), these dose reductions allowed toxic effects to disappear or diminish in grade to an acceptable level, thus permitting 10 patients (43%) to receive increased doses thereafter. For 1 patient experiencing severe nausea and vomiting and 1 with a grade 3 herpes zoster infection treatment was stopped by the physicians. No treatment-related deaths were observed.

The planned dose intensity of the treatment was 462.5 mg/m^2 5-FU per week. Patients treated for at least three cycles received a mean dose intensity of 434.4 ± 76.4 mg/m^2 per week (S.D.) 5-FU, which corresponded to 93.9 ± 16.5% of the planned dose intensity.

Table 3. Toxicity in 79 evaluable patients

Toxic effect*	WHO grade 1	2	3	4	Total (%)
Mucositis	25	21	9	3	58 (73)
Diarrhoea	15	14	14	0	43 (54)
Nausea/vomiting	26	8	4	1	39 (49)
Leucopenia	4	7	1	1	13 (16)
Alopecia	7	5	1	0	13 (16)
Cutaneous toxicity†	8	2	3	0	13 (16)
Conjunctivitis	7	2	1	0	10 (13)
Asthenia	8	1	0	0	9 (11)
Abdominal pain	7	1	0	0	8 (10)
Infection	3	2	1	0	6 (8)
Cardiotoxicity‡	3	0	1	0	4 (5)
Thrombocytopenia	2	0	0	0	2 (3)
Ageusia	2	0	0	0	2 (3)
Peripheral neurotoxicity	0	1	0	0	1 (1)
Phlebitis	1	0	0	0	1 (1)

* Correlation with treatment = certain, possible, probable. † 7 rash, 1 hyperpigmentation, 3 dermatitis, 1 rash + hand–foot syndrome, 1 hyperpigmentation + dermatitis. ‡ 2 tachycardia, 1 precordial pain, 1 atrial fibrillation.

DISCUSSION

Several phase II [1, 2] and phase III [3–12] studies of 5-FU combined with *d,l*-FA for the treatment of advanced colorectal carcinoma have been conducted. In these trials, the doses of the two drugs and their schedules and modalities of administration have largely varied, with response rates ranging from 16 to 48%. Until now investigators have not been able to define the regimen yielding optimal antitumour activity.

In the present study, we used a 5-day regimen comprising of a dose of the *l* isomer of FA (100 mg/m^2) equivalent to that given in the standard Machover regimen employing *d,l*-FA [17]. In the experience of Machover and colleagues [34], this dose allows plasma levels of active folates (*l*-FA and its metabolite 5-methyltetrahydrofolate) similar to those previously reported after rapid i.v. administration of *d,l*-FA at the dose of 200 mg/m^2 [17]. Similar equivalence has been demonstrated between the pharmaceutical preparation of pure *l*-FA (half dose) and the chiral *d,l* mixture of FA with short-term [35] and long-term [18, 36] i.v. infusion.

At the 100 mg/m^2/day *l*-FA dose [34], the concentrations of *l*-FA and folate metabolites in the central compartment are maintained for a sufficient time (up to 12 h) within the range (≥1 μM) required for maximal potentiation of the fluoropyrimidines *in vitro* [1, 2].

These parameters (i.e. concentration and time) play an important role in both the expansion and retention of intracellular folate pools involved in thymidylate biosynthesis [37–39], thus inducing an enhanced cytotoxicity of concurrently administered 5-FU.

The overall incidence of toxic effects in our patients was high. At least one episode of stomatitis, diarrhoea, or nausea/vomiting occurred in 73, 54 and 49% of the patients, respectively. Leucopenia, alopecia, cutaneous toxicity and conjunctivitis were experienced with incidence varying from 13 to 16%.

The degree of toxicity was mild to moderate in most cases (61%). The dose-limiting toxic effects observed were grade 3–4 diarrhoea, mucositis and nausea/vomiting which occurred in 18, 15 and 6% of patients, respectively. These could be controlled by reduction in the 5-FU doses during the following cycles in most patients.

Toxic effects in this study appear to be somewhat more frequent and severe than those reported by Machover and colleagues [34], but equivalent to those reported with similar 5-FU daily doses given in combination with double amounts of *d,l*-FA [17].

A possible explanation for this difference in toxicity might be related to the administration of 5-FU by i.v. infusion for 2 h used in the last Machover study [34] as opposed to a bolus (5–15 min) i.v. infusion employed previously by Machover and colleagues [17] and by us in the present study. Preliminary results of a phase II study by Creaven and colleagues [35] using a 1-day weekly regimen of a higher dose of *l*-FA (250 mg/

m^2) equivalent to that given in the standard RPMI regimen employing *d,l*-FA [40], by short term (2 h) i.v. infusion with 5-FU doses in the range of 600–750 mg/m^2 showed more frequent grade 3–4 diarrhoea and leucopenia, but less frequent mucositis compared to our study and that of Machover [34]. Toxicity patterns in this study were similar to those obtained by Madajewickz and colleagues [40] using the identical weekly regimen with similar 5-FU doses and double doses of *d,l*-FA.

Newman and colleagues [36] in a phase I trial using a 5-day continuous infusion *l*-FA and bolus 5-FU observed toxicities not differing in nature or severity from those produced by 5-FU and *d,l*-FA when administered on the same schedule [18]. Additional studies on the causes of the differences in toxicity, including duration of infusion of 5-FU, are warranted.

The response rate observed in this study was 15.5% (complete response 3%; partial response 12.5%; confidence limits 7–24%).

This result is within the range observed in previous studies of 5-FU in combination with *d,l*-FA, although at the lower level [3–13]. In the earlier phase II studies [1, 2] the mean response rate was 24% and in the more recent phase III studies it was 23% (range 16–48%) [13]. In these comparative trials, 5-FU alone obtained an average response rate of 11% (range 7–18%). The response rate observed in a clinical trial by Erlichman and colleagues performed using a comparable schedule of *l*-FA and 5-FU administration was similar (19%) [41].

The reduced efficacy of this combination in our study as well as in that of Erlichman and colleagues [41] might be related to the rapid i.v. administration modality of *l*-FA, which might have limited the time-dependent expansion of intracellular 5,10-methylenetethrahydrofolate pools, as demonstrated in experimental tumour models [37, 39]. However, by using the same dose and administration schedule of *l*-FA, Machover and colleagues [34] observed better therapeutic results (52% objective response rate). This was obtained with a slower (2 h) intravenous infusion of 5-FU rather than with a rapid i.v. bolus (5 min).

Preliminary results in 19 patients using a short-term (2 h) i.v. infusion of a higher dose of the folate and a 1-day weekly administration schedule [35] also showed higher response rate (47%). Plasma pharmacokinetic parameters of *l*-FA and 5-FU showed large interpatient variability but were not correlated with response.

Varying response rates among these studies might also be explained by differences in performance status and/or disease extension since these factors appear in our experience to affect, although not significantly, sensitivity to 5-FU. Our observations are in accordance with recent results in the literature: performance status has, in fact, been identified as a major determinant of response to 5-FU and FA in patients with metastatic colorectal cancer [42].

Our results suggest only a minor potentiation of 5-FU effectiveness by *l*-FA in patients with advanced colorectal carcinoma with the schedule employed. However, encouraging results with other administration modalities reported in phase II trials [34, 35] justify a more complete exploration of the administration modalities of the pure active stereoisomer as a modulator of 5-FU. Also the demonstration of a difference in the effectiveness of 5-FU given in combination with each of the two pharmaceutical formulations of FA requires further comparative clinical studies with larger numbers of patients using active administration schedules.

Recent experimental data in an *in vitro* tumour model have indicated a modulatory action of fluoropyrimidine cytotoxicity by high concentrations (10–100 μM) of the unnatural *d* isomers of both FA and 5-methyltetrahydrofolate whose mechanisms are not yet clear, but may involve direct effects at the thymidylate synthase level on the formation on the ternary complex [43].

Thus, on the basis of the clinical results, as well as of this preliminary experimental evidence, the need also emerges for a more detailed evaluation of the pharmacological effects of the pure isomers of FA in order to determine their optimal therapeutic use.

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