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ORIGINAL ARTICLE

M. Boisdron-Celle · M.C. Craipeau · S. Brienza
R. Delva · V. Guérin-Meyer · E. Cvitkovic · E. Gamelin

Influence of oxaliplatin on 5-fluorouracil plasma clearance and clinical consequences

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Abstract The influence of oxaliplatin (OXA) on 5-fluorouracil (5-FU) plasma clearance was investigated. *Patients and methods:* A group of 29 patients with advanced colorectal cancer refractory to prior weekly 8-h 5-FU infusion plus bolus folinic acid (FA), received the same combination plus OXA at 130 mg/m^2 every 3 weeks, OXA plus 5-FU plus FA on day 1, and 5-FU plus FA on days 8 and 15. Steady-state 5-FU concentrations in plasma were measured weekly and 5-FU clearance was calculated. Both before and after the addition of OXA, the 5-FU dose was individually adjusted according to the pharmacokinetic follow-up (target steady-state plasma concentrations 2.5–3 mg/l). *Results and discussion:* A total of 122 OXA-containing infusions and 338 5-FU plus FA infusions were given and the median number of infusions per patient was 4 (2–9) and 10 (5–28), respectively. 5-FU plasma clearance was significantly decreased on days 8 and 15 when compared with the value on day 1 and with the values before OXA introduction using a direct paired comparison (2.36 and 2.31 l/min, respectively, vs 3.12 and 3.05 l/min; $P < 10^{-5}$). Of 25 evaluable patients, 6 had an objective response after the introduction of OXA (24% objective response rate, 95% confidence interval 9.4–45%). *Conclusion:* OXA reduces 5-FU plasma clearance for 15 days. This may be a factor in the synergy between the two drugs. It is not linked to dihydropyrimidine dehydrogenase inhibition. Implications for drug schedules in clinical practice are discussed.

Keywords Fluorouracil · Oxaliplatin · Pharmacokinetics · Interference

M. Boisdron-Celle · M.C. Craipeau · R. Delva
V. Guérin-Meyer · E. Gamelin (✉)
Department of Medical Oncology and
Clinical Pharmacology, Centre Paul Papin,
Centre Régional de Lutte Contre le Cancer,
49000 Angers cedex, France
E-mail: e.gamelin@unimedia.fr
Tel.: +33-41-352700
Fax: +33-41-483190

S. Brienza
Debiopharm France, Charenton Le Pont, France

E. Cvitkovic
Department of Medical Oncology, C.H.U. Paul Brousse,
94800 Villejuif, France

Introduction

Oxaliplatin (OXA) is a diammine cyclohexane platinum complex, active in several solid tumour types, including some cisplatin/carboplatin-refractory diseases such as advanced colorectal cancers [17, 20]. In combination with 5-fluorouracil (5-FU) and folinic acid (FA), the objective response rate is usually around 50% in first-line treatment of advanced disease, with a median survival time ranging from 12 to 17 months [1, 17, 18]. The addition of OXA can reverse 5-FU clinical resistance in patients with advanced colorectal cancers refractory to 5-FU plus FA, allowing a response rate ranging from 22% to 46%, whereas alone it provides an objective response rate of 10% [3, 17]. Several in vitro and in vivo studies have shown that OXA elicits a supraadditive effect or a high potentiation of efficacy when combined with 5-FU [22].

The pharmacokinetics of OXA have been assessed, either after single or repeated doses of 130 mg/m^2 every 3 weeks [11]. 5-FU has been used in various administration schedules. Both short-term and continuous 5-FU infusion pharmacokinetics have been investigated [4, 26]. With constant infusion, 5-FU plasma clearance is rapid, but decreases with increasing dose because metabolism is saturable [2, 21]. 5-FU is first transformed into its dehydrogenated inactive metabolite, dihydrofluorouracil, by dihydropyrimidine dehydrogenase (DPD) and then further transformed into α-fluoro-β-ureidopropionate, then fluoro-β-alanine [24, 26]. The incidence of 5-FU toxicity increases with higher systemic exposure, i.e. steady-state plasma concentrations with constant infusion, whatever the administration schedule [29, 31]. Individual dosage adjustment with pharmacokinetic monitoring helps to prevent toxicity [5, 30].

A relationship between 5-FU plasma levels and tumour response has been reported [15, 28, 32]. We have previously carried out a first multicentric prospective trial in patients treated with individually increasing 5-FU doses in a weekly 5-FU plus FA 8-h continuous intravenous (i.v.) infusion scheme. A pharmacokinetic follow-up allowed us to show a relationship between 5-FU plasma concentrations and therapeutic outcome, both in terms of treatment tolerability and efficacy [8]. Our data suggested that plasma levels higher than 2.5 mg/l (efficacy threshold) and lower than 3.0 mg/l (toxic threshold) provide the best safety:efficacy ratio. We set up a chart for individual 5-FU dose adjustment according to a weekly pharmacokinetic follow-up [8]. Then, in a second multicentric prospective trial carried out in 152 patients, we showed the advantage of individual dose adjustment with pharmacokinetic monitoring in terms of treatment tolerability and efficacy [12].

In the present monocentric open-label study, we introduced OXA into the weekly 5-FU schedule in the treatment of patients with advanced colorectal cancer refractory to 5-FU plus FA while on treatment. We investigated the possible pharmacokinetic-pharmacodynamic influence of OXA on 5-FU. The primary objective was to study the influence of OXA on 5-FU plasma levels, by comparing the levels before and after OXA introduction to the weekly 5-FU plus FA regimen. The secondary objective was to elucidate pharmacodynamic interactions in terms of the differential incidence of 5-FU-related toxicities before and after the addition of OXA.

Patients and methods

Study design

Patients had advanced adenocarcinoma of the colon or the rectum with a pathologically confirmed diagnosis, treated with a 5-FU plus FA weekly combination at an individually adjusted dose. Upon disease progression, OXA was added at the standard recommended dose and schedule of 130 mg/m^2 every 3 weeks to the 5-FU plus FA weekly regimen.

To be eligible, patients had to have a performance status (PS) ≤ 2 according to the World Health Organisation classification, a life expectancy of at least 3 months, an age less than 75 years, an adequate haematological status and at least one measurable metastasis in a non-irradiated area. Brain metastasis and a cardiac condition contraindicating 5-FU treatment were non-eligibility criteria. All patients had normal liver function, all except two had normal renal function (creatinine clearance >65 ml/min per 1.73 m^2), and none had signs of ascites or pleural effusion.

Written informed consent from all the patients and relevant Ethical Committee approval were obtained before starting the trial.

Treatment

Before the OXA study, patients were treated with 5-FU and FA in a weekly schedule with dose adjustment to be in the therapeutic range (i.e. 2.5–3 mg/l) as previously reported [11]. After progression on this regimen, the treatment schedule consisted of the same regimen plus OXA: day 1 OXA 130 mg/m^2, 2-h infusion in 250 ml sterile 5% glucose solution, then days 8 and 15 5-FU and FA. Treatment was restarted on day 21.

5-FU was planned to be given at the same infusion rate as previously given before introducing OXA into the combination, in order to keep unchanged the 5-FU-based regimen under which progressive disease was documented. 5-FU was delivered as an 8-h infusion in 1 l 0.9% saline serum via a battery-operated pump, and infusion was started at 10.30 a.m. FA was given as an i.v. bolus of 400 mg/m^2 just before and 4 h after starting the 5-FU infusion. Treatment was delivered through a long-term venous access. Ondansetron and corticosteroids were given as prophylactic antiemetics before OXA administration. Treatment was prolonged until tumour progression unless severe toxicity occurred or the patient refused further treatment.

The OXA dose was maintained unchanged in all patients, while cycle delays of 1 to 2 weeks occurred for incomplete haematological recovery or acute neuropathy symptoms. In these situations, 5-FU was continued weekly until OXA reintroduction. 5-FU dose adjustment was performed according to 5-FU plasma assays and to a table of 5-FU dose adjustment, designed with the purpose of maintaining steady-state concentrations between 2.5 and 3.0 mg/l above which increased incidences of diarrhoea, mucositis and hand-foot syndrome toxicities have been shown to occur [8].

Clinical assessments

Clinical toxic events, especially gastrointestinal tract toxicities, mucositis, hand-foot syndrome and neurotoxicity, were evaluated weekly and graded following NCI-CTC scales, except neurotoxicity, which consisted of selected neurotoxic symptoms characteristic of OXA administration (cryodysaesthesia, cramps, laryngopharyngeal spasm and functional impairment). Haematological and biochemical toxicities were evaluated every 3 weeks before the next OXA infusion.

The efficacy end-point was response rate. The assessment of tumour response was performed after three complete cycles from the initiation of the treatment and then every 2 months. Response to treatment was classified according to WHO criteria [23]. An independent board to assess tumour response reviewed CT scans of all patients. The duration of response was also calculated (from 3 months of treatment to the time of disease progression).

Pharmacokinetics

5-FU plasma levels were measured in all patients while under treatment with the prior 5-FU/FA regimen and after introducing OXA into the same combination. 5-FU plasma clearance was calculated as follows [27]: 5-FU plasma clearance = 5-FU infusion rate/5-FU plasma concentration at steady-statee, where 5-FU infusion rate is 5-FU dosage/infusion duration (in milligrams per hour) and 5-FU plasma concentration is in milligrams per litre. The 5-FU plasma concentration measured 7 h after the start of 5-FU infusion was considered as the steady-state since the half-life of 5-FU is usually 10–15 min [4, 26].

Blood sampling

Blood samples of 5 ml were collected into heparinized tubes during the hour before the end of 5-FU infusion, to ensure that steady-state conditions prevailed, on day 1, day 8 and day 15 of each 3-week cycle. The plasma was immediately separated by centrifugation at 4ºC in the hospital ward and was stored at –20ºC until analysis. 5-FU concentrations in plasma were determined by high-pressure liquid chromatography as previously described [9]. In summary, 5-FU was extracted from plasma with isopropanol/ethyl acetate (85/15 v/v) in the presence of 200 mg ammonium sulphate to precipitate proteins. The organic phase was dried at 56ºC under nitrogen. The mobile phase was potassium phosphate solution (KH_2PO_4 10 mmol/l). HPLC was carried out using a Spherisorb ODS1 column. and UV detection was performed at 260 nm. The chromatograms were treated with a PC integrator. The limit of quantification was 6 ng/ml.

Simultaneous concentrations in plasma of uracil, 5-FU and their dehydrogenated metabolites, 5-dihydrofluorouracil (5-FUH_2) and dihydrouracil (UH_2), were also measured according to a previously reported method [10]. HPLC was carried out using serially mounted Spherisorb ODS1 and ODS2 columns, and 10 m*M* phosphate buffer, pH 3.0, as the mobile phase with UV detection at 205 nm. The chromatograms were treated with a PC integrator. The limit of quantification was 6 ng/ml. We calculated the UH_2:uracil and 5-dihydrofluorouracil:fluorouracil ratios. The second method, which took longer than the first one, was used only for the simultaneous determination of the four pyrimidines but, in current practice, the first method is usually used for 5-FU measurement in plasma.

Statistical study

Data analysis and statistical study

All clinical, radiological and laboratory data were retrospectively controlled by a Trial Monitor and Clinical Research Assistants. The statistical analyses were undertaken using Statistica 5.1 (Statsoft) and SPSS 61 (SPSS) on a Windows 95 platform.

The statistical methods employed consisted of:

- Descriptive statistics: mean, median, range of values, 95% confidence interval and percentages, for the description of demographics, pretreatment and treatment characteristics, and toxicity profiles, as well as elements of the analysis of the pharmacokinetic interaction, the relationship between toxicity and plasma levels and the influence of OXA on 5-FU-specific toxicities.
- Direct-paired comparison, each patient being his/her own control. Student's paired *t*-test was used to compare 5-FU plasma clearance before and after OXA introduction.
- Analysis of variance: ANOVA modelling of 5-FU plasma clearance, considering the OXA cycle and the day of 5-FU infusion (days 1, 8, 15) as two repeated factors and the 5-FU dose as covariate, with no between factors,
- Tests for correlation: Pearson's, *t*-test and *F*-test for the determination of the degree of correlation between 5-FU-specific toxicity and 5-FU plasma levels and the influence of OXA on the incidence of 5-FU-specific toxicities.

Parameters analysed

Pharmacokinetic interaction. 5-FU plasma clearance was compared before and after OXA introduction. Before OXA introduction, 5-FU plasma levels were recorded during the last two or three 5-FU infusions. After OXA introduction, only complete cycles and their corresponding 5-FU plasma concentrations were taken into account for analysis, i.e. those cycles in which 5-FU was given on day 1, in conjunction with OXA, followed by 5-FU plus FA on days 8 and day 15. Only 5-FU concentrations during the hour before the end of 5-FU infusion were measured and plasma clearance calculated. Thus, circadian rhythm in 5-FU metabolism did not interfere.

The analysis thus compared the 5-FU plasma clearance before the introduction of OXA with those of the complete first cycles of OXA plus 5-FU and FA combination treatment, and with those of all complete cycles.

Toxicity. In contrast, toxicity was analysed for the last documented 5-FU plus FA monotherapy infusion and for all cycles, whether complete or not, of OXA plus 5-FU and FA. Toxicity was reported according to cycle and the maximum grade experienced by patients during treatment with OXA. Given that clinical toxicities were reported weekly, toxicity per cycle was taken to be the maximum grade reported for a patient during the three 5-FU plus FA infusions on days 1, 8 and 15 of the OXA cycle.

The relationships between 5-FU concentrations in plasma and selected toxic events were investigated. The toxicities included haematological toxicity, digestive tract toxicity, cutaneous toxicity and all of these toxicities combined. Only patients whose 5-FU dose, 5-FU concentrations and toxicity were documented for the last three weekly 5-FU infusions were analysed for relationship between 5-FU concentrations and toxicity under prior 5-FU plus FA treatment. All complete cycles under OXA plus 5-FU and FA treatment the toxicity of which was reported and for which 5-FU dose and concentrations were available were analysed.

Results

A total of 29 patients with 5-FU-refractory metastatic colorectal cancer entered the study between June 1997 and April 1998. Their characteristics are displayed in Table 1. They were in a generally good condition at inclusion (PS $\leq$ 1). A total of 122 complete OXA-containing cycles and 338 5-FU plus FA infusions were administered. Table 2 details the characteristics. The initial 5-FU dose for each patient was based on the last doses administered at the end of the previous regimen with 5-FU plus leucovorin. These 5-FU doses were those that had provided a therapeutic 5-FU level (2.5 to 3.0 mg/l). They displayed a wide interpatient variability (1750 ± 570, range 750 to 3500 mg/m^2 per week).

Clinical results

Data on toxicity occurring during the weekly 5-FU plus FA pretreatment were evaluated and reported for 27 out of the 29 patients analysed, and for all the 29 patients and 122 OXA plus 5-FU plus FA combination treatment cycles. The WHO toxicity grading system was used for all toxicity types, except for OXA-induced neurotoxicity, which has specific symptoms. The toxic manifestations reported in the two successive combinations are presented in Table 3.

Only two cycles of grade IV toxicity in two patients (thrombocytopenia) were reported, and grade III toxic events occurred only occasionally, except diarrhoea which occurred in ten cycles (8% of cycles) and in seven patients. Distal paraesthesia, induced or exacerbated by cold, were experienced by 22 patients (76%), over half the OXA infusions given. Other symptoms such as laryngopharyngeal dysaesthesia and cramps were uncommon (reported on two and four occasions, respectively). Neurotoxicity resulted in functional impairment in seven patients (24%) who had received a median cumulative OXA dose of 390 mg/m^2. Four patients experienced mild to moderate infectious episodes.

Pharmacological results

All patients were evaluable for 5-FU concentrations before OXA introduction, and 27 of these received at least one complete OXA cycle. All OXA treatments were

Table 1 Clinical and biological characteristics of the 29 patients

Sex		
Male	16	55%
Female	13	45%
Age (years)		
Median	64	
Range	31–73	
Primary tumour		
Colon	11	38%
Rectum	13	45%
Rectosigmoid	5	17%
No. of 5-FU + FA weekly treatments before OXA		
Total	730	
Median	25	
Range	1–48	
Weekly 5-FU dosage given before OXA (mg/m^2)		
Median	1750	
Range	200–3360	
Interval between last 5-FU + FA cycle and inclusion (weeks)		
Median	6	
≤ 4	12	41%
5–12	10	34%
> 12	7	24%
No. of lines of previ-ous chemotherapy		
1	23	79%
2	4	14%
3	2	7%
Performance status (WHO criteria)		
0	12	46%
1	13	50%
2	1	4%
Missing	3	
Abnormal baseline laboratory values		
Haemoglobin < 9 g/dl	0/28	0%
Neutrophils < 2000/mm3	1/29	3%
Liver function tests	9/25	36%
Renal function	1/26	4%

Table 2 Characteristics of the treatments

OXA	Total number of infusions	122
	Number of infusions/patient	
	Median	4
	Range	2–9
	Dose/infusion (mg/m^2)	
	Median	130
	Range	125–143
	Cumulative dose/patient (mg/m^2)	
	Median	510
	Range	260–1125
	Number of cycles delayed	
	No delay	76
	Delayed 1 week	11
	Delayed 2 weeks	1
	Delayed 3 weeks	5
	Total	93/122
5-FU + FA	Total number of treatments	338
	Number of treatments/patient	
	Median	10
	Range	5–28
OXA + 5-FU + FA	Total number of complete cycles given	82 (67%)
	Number of complete cycles/patient	
	Median	2
	Range	0–8

administered at the recommended dose of 130 mg/m^2 and given as a 2-h infusion.

We compared 5-FU plasma clearance before OXA introduction, i.e. 5-FU plasma clearance baseline or on day 1, with both 5-FU plasma clearance on day 8 and day 15. We found that OXA reduced 5-FU plasma clearance for 15 days (Fig. 1). The difference according to a direct paired comparison test was highly significant for the whole population of patients. Similar results were obtained when we compared the clearances of the last three infusions of 5-FU with those recorded in all the complete OXA cycles. In an additional analysis, taking as baseline all 5-FU clearances in plasma recorded before OXA introduction ($n = 730$, mean number of 5-FU infusions per patient 25, range 1–48), the results (data not shown) were superimposable on those presented above, showing that no bias was introduced by the selection of the baseline 5-FU clearances used in the above analysis. Accordingly, the ANOVA test gave equivalent results ($P < 1 \times 10^{-6}$ for all cycles).

The time-course of OXA-induced increased 5-FU plasma levels was accurately described in the three patients in whom OXA administration was postponed until day 29 or 36 for haematological toxicity. A representative example from these three patients is given in Fig. 2. In this patient, who received stable 5-FU doses, a marked increase (+33%) in 5-FU plasma levels was observed on day 8 and was maintained on day 15. On day 22, when the new OXA dose to be given had to be delayed, 5-FU levels were almost similar to baseline values (+9%) and to those recorded 8 days later on day 29. The duration of the interaction between OXA and 5-FU kinetics is further illustrated by the similarity in 5-FU plasma levels observed in those patients while treated with 5-FU/FA alone (Table 4).

Relationship between toxicity and 5-FU plasma concentrations

The increased 5-FU plasma concentrations on day 8 and day 15 were followed by an increased incidence of diarrhoea, hand-foot syndrome and mucositis when they reached 3000 μg/l. The histogram shown in Fig. 3 shows the distribution of the 32 toxic events according to the 5-FU levels in plasma. For the whole population of patients, the relationships between 5-FU plasma concentrations and toxicity were investigated for every toxicity type combined. They were graded as the worst grade experienced during the last three 5-FU plus FA and complete OXA cycles, documented in terms of dose, 5-FU plasma concentrations, and toxicity. A total of 16 patients were evaluable for 5-FU plus FA alone and 27 for OXA plus 5-FU plus FA treatment, over 79 complete cycles. Due to the small number of toxic events, the analysis involved a comparison of cycles without toxicity

Table 3 Number and percent of cycles with toxicity. Values in parentheses refer to cycles with toxicity observed in the previous treatment (*NA* not available)

WHO toxicity type	Number of cycles with toxicity grade					Percent of cycles with toxicity grade		
	0	I	II	III	IV	0	I+II	III+IV
Haematological								
Anaemia	100 (25)	16 (2)	5 (0)	1 (0)	0 (0)	82 (93)	17 (7)	1 (0)
Leucopenia	100 (25)	12 (2)	8 (0)	1 (0)	0 (0)	83 (93)	16 (7)	1 (0)
Thrombocytopenia	100 (27)	7 (0)	7 (0)	0 (0)	2 (0)	87 (100)	11 (0)	2 (0)
Gastrointestinal								
Nausea, vomiting	97 (24)	12	11	0	0	80 (89)	19 (11)	2 (0)
Diarrhoea	66 (24)	24	22	10 (7)	0	54 (89)	38 (7)	8 (4)
Mucositis	117 (26)	1	2	0	0	96 (96	4 (4)	0 (0)
Cutaneous	98 (15)	8	3	1	0	80 (56)	20 (41)	0 (4)
Neurotoxicity								
Cryodysaesthesia	59 (26)	63 (1)	NA	NA	NA	48 (96)	52 (4)	NA
Laryngopharyngeal dysaesthesia	120 (27)	2 (0)	NA	NA	NA	98 (100)	2 (0)	NA
Cramps	118 (27)	4 (0)	NA	NA	NA	97 (100)	3 (0)	NA
Function impairment	105 (26)	17 (1)	NA	NA	NA	86 (96)	14 (4)	NA
Other toxicities								
Infection	26	0	1	0	0	97 (96)	4	0 (0)
Fever	27	0	0	0	0	99 (100)	3 (0)	0 (0)

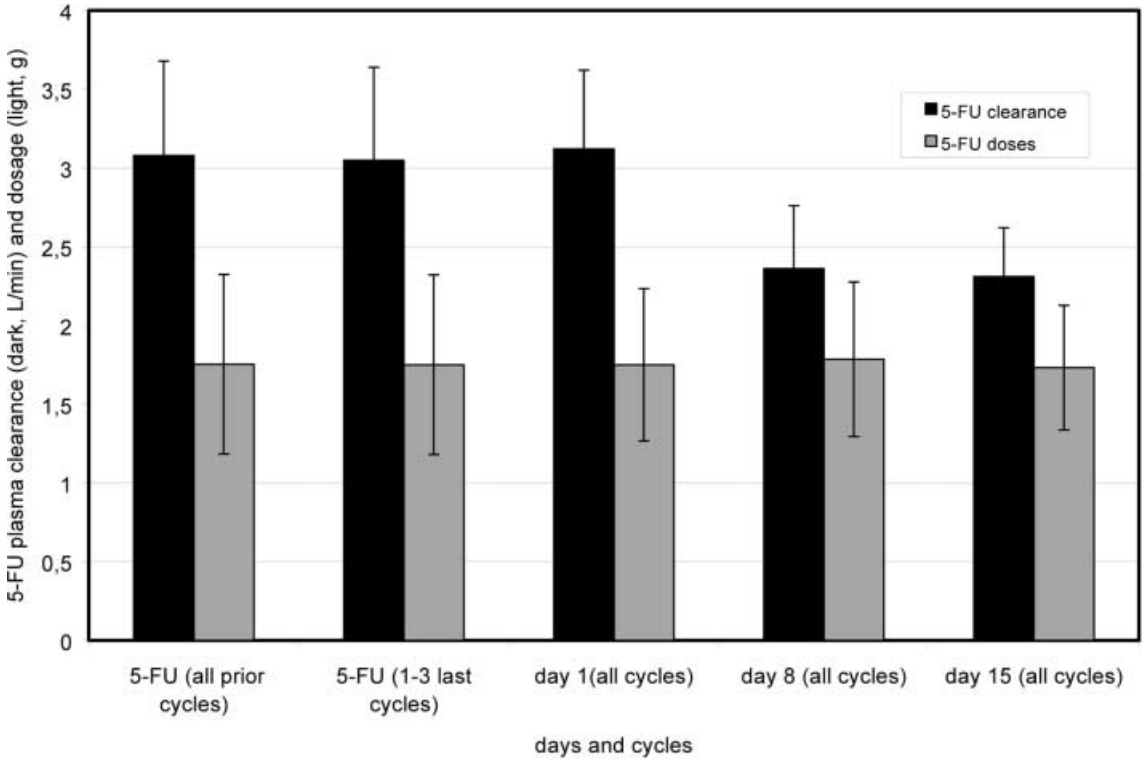

Fig. 1 5-FU-plasma clearance and 5-FU dose before and with OXA (all prior cycles; one to three last cycles; days 1, 8, 15)

and cycles with any grade of toxicity. In addition, another analysis was performed, considering the incidence of toxic events in cycles in which the mean 5-FU plasma concentrations were higher or lower to 3000 μg/l.

There was a significant relationship between the 5-FU plasma levels and the mean or maximum 5-FU plasma levels and the occurrence of toxicity. The mean 5-FU level was significantly higher (2.73 ± 0.47 vs 2.46 ± 0.52 mg/l, $P = 0.046$) in cycles in which toxicity occurred (62 cycles vs 17 cycles). Similar conclusions were reached when 5-FU plasma levels above and below 3.0 mg/l were considered for differential rates of toxicity.

Comparison of toxicity under 5-FU plus FA monotherapy and OXA plus 5-FU and FA combination

The possible pharmacodynamic consequences of the pharmacokinetic interaction between OXA and 5-FU

were investigated. The toxicity data recorded during the first OXA cycle were compared with those recorded during the last three 5-FU plus FA treatments. A total of 16 patients were evaluable for toxicity, for both the last three 5-FU infusions and the 5-FU and OXA combination. The toxicity data provided no evidence of potentiation of 5-FU toxicity by OXA (Table 3), since the incidence of 5-FU-specific toxicities, i.e. mucositis and cutaneous toxicity, remained unchanged after the introduction of OXA.

Antitumor efficacy

The overall response rate in 27 patients was partial response in 7 patients, stable disease in 12 patients and progressive disease in 8 patients; 2 patients could not be evaluated. Thus, the objective response rate reported for the evaluable patients in this cohort was 24%. Median time to progression was 9 months (0 to 16 months).

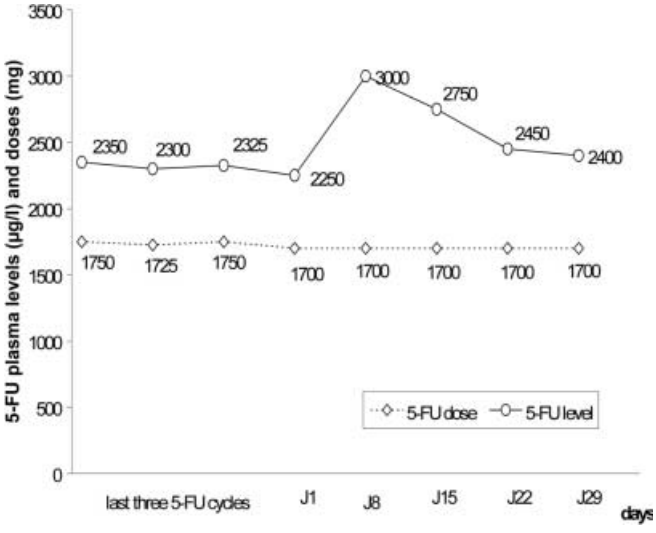

Fig. 2 Time-course of OXA-induced increased levels of 5-FU in one representative patient with stable 5-FU dose, in whom the next course of OXA had been postponed for 2 weeks, whereas 5-FU had been continued weekly. From day 15, 5-FU plasma levels decreased down to levels similar to those on day 1

Uracil and dehydrogenated metabolite levels

Endogenous pyrimidine, uracil and the dehydrogenated metabolites, UH_2 and 5-FUH_2, were also measured in plasma. Uracil levels in plasma followed those of 5-FU. They had increased significantly on days 8 and 15 compared with day 1. Plasma levels of both UH_2 and 5-FUH_2 also increased so that the ratio of dehydrogenated metabolites to pyrimidine substrate remained unchanged. Interestingly, the mean dihydrouracil plasma levels were exactly equivalent to those of uracil and their ratio was 1. The ratio of 5-FUH_2 to 5-FU was around 0.5, i.e. half the ratio of UH_2 to uracil (Table 5).

Discussion

Under the experimental conditions of this trial, the results provide evidence for a significant influence of OXA on 5-FU plasma clearance, recorded under a weekly 5-FU schedule when OXA was given every 3 weeks. OXA had reduced 5-FU plasma clearance on day 8 and day 15. Its influence probably lasted 15 days and ended between day 15 and day 22. This observation was confirmed in three patients whose OXA administration was postponed until day 29 or 36 because of an OXA-induced thrombopenia, and who had a progressive decrease in 5-FU plasma levels from days 8 and 15 to days 22, 29 and 36. 5-FU plasma concentrations then became equivalent to those on day 1, just after OXA infusion.

The interaction found when baseline 5-FU levels were limited to the last one to three 5-FU infusions given before the introduction of OXA was confirmed when all the available 5-FU levels recorded in the patients during their prior weekly 5-FU treatment were analysed. Thus, no bias was introduced into the analysis by the selection of a limited number of 5-FU baseline plasma levels. In addition, the validation studies allowed interference of OXA in the 5-FU assay method to be excluded, and methodological bias introduced by the administration of ondansetron on day 1 of each cycle but not on day 8 or 15 also to be excluded (data not shown). We used two statistical tests, direct paired comparison and ANOVA models.

Table 4 5-FU plasma concentrations before and with OXA addition

	Mean dose (mg)	SE	Student's t-test day x vs day 1	*P*-value	Mean plasma level (µg/l)	SE	Student's t-test day x vs day 1	*P*-value
Before OXA								
All prior 5-FU cycles	1753	570			2370	228		
One to three last 5-FU cycles	1750	570			2390	228		
With OXA								
Day 1	1755	483			2300	383		
Day 8	1785	491	1.79	NS	3121	299	5.30	$< 10^{-3}$
Day 15	1732	396	–1.36	NS	3200	532	5.38	$< 10^{-3}$

Our results are in agreement with those of Papamichael et al. who treated ten patients in a crossover study with the biweekly de Gramont regimen without and then with OXA. OXA did not influence 5-FU pharmacokinetics during the 2 days that followed OXA administration [13, 25]. We found equivalent results during the hours following OXA infusion. In fact, the interference was delayed, suggesting an action on catabolism or anabolism gene expression.

The relationship between 5-FU concentrations and the most frequent toxicity types, including diarrhoea, hand-foot syndrome and mucositis, found when the 5-FU plus FA weekly schedule was given alone [8, 12], persisted in the current study. We found again that the incidence of all 5-FU-related toxic events was increased in cycles in which the mean levels of 5-FU were above 3.0 mg/l, this situation being more often reached on days 8 and 15. These results indicate the advantage of individual 5-FU dose adjustment, especially when a 5-FU dose intensification is planned, in combination with OXA since 5-FU catabolism is saturable and OXA reduces it [2]. The other toxic events, more OXA-related, were generally mild, with only two episodes of grade 4 thrombocytopenia and 16 cycles with grade 3 toxicity (13% of cycles). However, as usually observed with OXA, the majority of patients (22/29) experienced cryodysaesthesia, and neurotoxicity resulted in functional impairment in seven patients (24%).

Among the 27 patients (a population of patients refractory to a 5-FU plus FA schedule) evaluated for tumour response, 7 showed a partial response and 12 stable disease. It is worthy of note that the clinical results in terms of tumour response and patient survival are equivalent to those previously reported [3]. Thus, 5-FU dose adjustment leads to a reduction in the

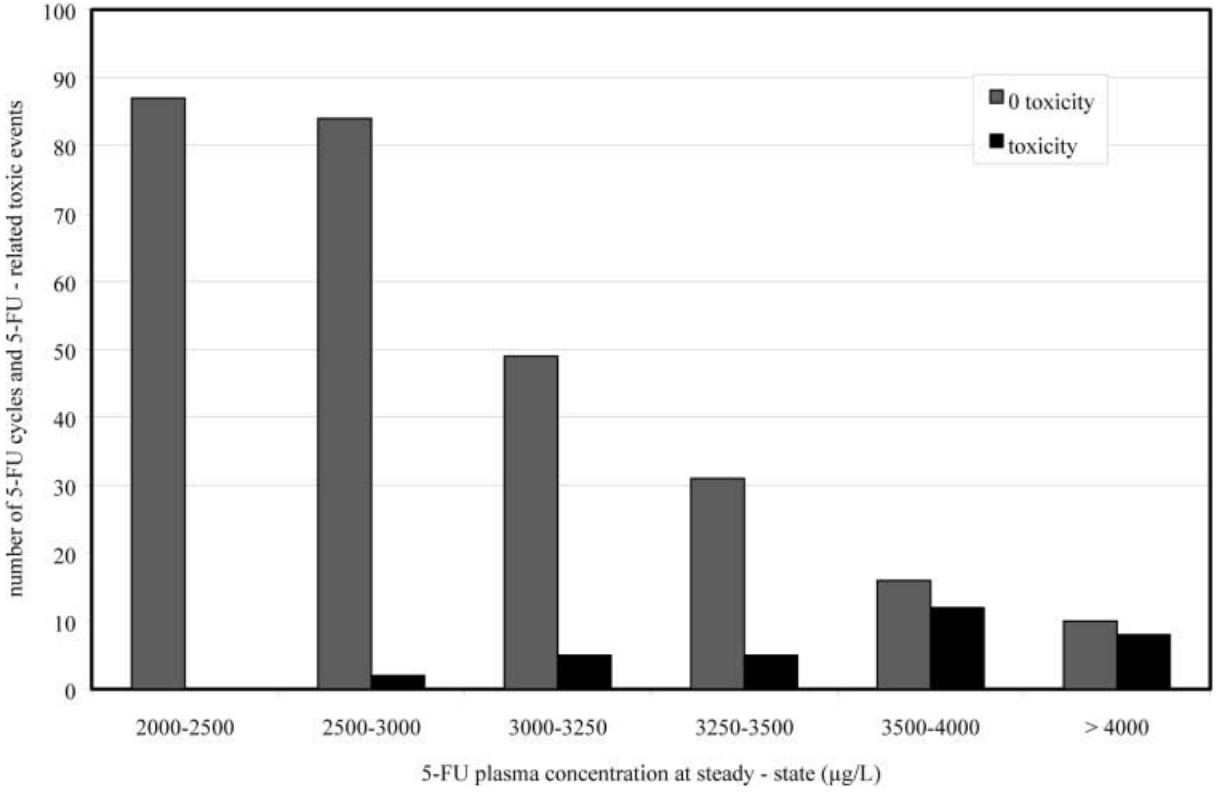

Fig. 3 5-FU plasma concentrations (micrograms per litre) before toxic side effects

Table 5 Plasma concentrations of 5-FU, uracil and their dehydrogenated metabolites on days 1, 8 and 15 after OXA administration (34 complete cycles, 15 patients; values are means ± SD). Values for days 8 and 15 were compared with those for day 1 (Student's paired test)

Day	5-FU		5-FUH_2		FUH_2:FU ratio	Uracil		UH_2		UH_2:uracil ratio
	µg/l	*P*-value	µg/l	*P*-value		µg/l	*P*-value	µg/l	*P*-value	
1	2288 ± 444		1307 ± 509		0.53 ± 0.2	90 ± 33		97 ± 42		1.085 ± 0.34
8	3116 ± 825	0.005	1663 ± 906	0.06	0.54 ± 0.31	129 ± 40	0.0049	130 ± 54	0.01	1.07 ± 0.39
15	3055 ± 595	0.005	1864 ± 1007	0.007	0.64 ± 0.36	143 ± 48	0.0042	150 ± 54	< 0.01	1.17 ± 0.46 (0.15)

incidence of toxic events and does not alter the clinical benefit in terms of response.

Therefore, the kinetic interaction found may be a factor in 5-FU and OXA potentiation, but the mechanism remains unclear. The interaction was not in protein binding since this compartment represents a very minor part of 5-FU in plasma [4, 26]. It was not mediated by a decrease in renal excretion. 5-FU renal excretion is a minor way of elimination and OXA has no effect on renal function [4, 20, 26]. OXA influenced not only 5-FU clearance, but also that of another pyrimidine, uracil whose concentrations in plasma had increased on days 8 and 15 after OXA administration. The first catabolic step performed by DPD [7, 14] has been investigated further and according to our current results, we can assume that DPD inhibition, or suppression of DYPD gene expression, was not the molecular mechanism of interaction. The ratios of dehydrogenated metabolites to the substrates, 5-FU and uracil, remained unchanged, whatever the time of plasma assay and the levels of the two substrates in the plasma. If OXA had indeed inhibited DPD, whatever the mechanism, we would have observed a fall in the dehydrogenated metabolites.

Two hypothetical mechanisms could account for the effect of OXA on 5-FU and uracil clearance. OXA could inhibit 5-FU catabolism downstream, for instance the dihydropyrimidinase, the second enzyme of the catabolic pathway. OXA could also inhibit the anabolic pathway, such as 5-FU incorporation into DNA or RNA, the action on thymidylate synthetase being the predominant mode of action. Recently, Fischel et al. have reported similar results from in vitro studies. They incubated cells with both 5-FU and OXA and found an increase in intracellular 5-FU and were able to show that DPD activity was not inhibited [6]. Their results are in favour of our hypothesis that OXA inhibits 5-FU catabolism, not via DPD, and thus would enhance its incorporation into RNA and DNA, by the inhibition of thymidylate synthetase, and this is the most important mechanism of 5-FU cytotoxicity.

The influence of OXA on 5-FU kinetics reported here could be a factor in the synergy reported in clinical studies in patients with 5-FU-refractory advanced colorectal cancer [3]. The objective response rate was about 22%, higher than that obtained in the equivalent group of patient treated with OXA alone. In clinical practice, the conventional scheme of biweekly OXA and 5-FU may not be the best combination, since the interaction is delayed and lasts about 15 days. Weekly OXA could be a more appropriate schedule. In the same way, the cytotoxic effect of 5-FU could be optimized by weekly administration such as in the AIO (Arbeitsgemeinschaft Internische Onkologie) regimen (weekly 24-h infusion) [16] or by continuous infusion such as in Lokich's regimen [19] during the 15 days following OXA infusion. The synergy between 5-FU and OXA could probably be optimized by new protocols more adapted to their reciprocal interaction and providing the best conditions for maximum potentiation.

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