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Gemcitabine increases systemic 5-fluorouracil exposure in advanced cancer patients

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Abstract

A number of recent clinical trials testing the combination of 5-fluorouracil (5-FU) and gemcitabine in patients with advanced pancreatic adenocarcinoma have shown a significant clinical response rate, but also significant toxicity. As the two antimetabolites may interact at several biochemical levels along their pathways of activation, we investigated whether gemcitabine (GEM) affects 5-FU pharmacokinetics in cancer patients. Thus, we compared 5-FU pharmacokinetics in two groups of patients with various cancers who received the same schedule of 5-FU and folinic acid (FUFA), with or without GEM. There was a significant increase in systemic (5-FU) exposure and toxicity in the FUFA plus GEM group. Our finding may be useful in designing future studies of the combination in order to reduce the occurrence of side-effects and to maximise the antitumour activity.
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1. Introduction

5-Fluorouracil (5-FU), one of the major components in the treatment of colorectal, breast, head and neck, and pancreatic carcinomas [1–3], is a pro-drug that needs to be converted into 5-fluoro-deoxyuridine-monophosphate (5FdUMP) and 5-fluoro-uridine-triphosphate (5FUTP) in cancer cells [1–3]. GEM (gemcitabine; difluoro-2′,2′-deoxycytidine) is a pyrimidine antimetabolite that is active against breast, pancreatic and biliary tract carcinomas [1–6]. It requires activation throughout the synthesis of its metabolites difluoro-2′,2′-deoxycytidine triphosphate (dFdCTP), which is incorporated in the DNA and inhibits chain elongation, and difluoro-2′,2′-deoxycytidine diphosphate (dFdCDP), which is also capable of inhibiting the activity of ribonucleotide reductase [3,4]. On the basis of the results of preclinical studies showing that 5-FU and GEM have synergistic antitumour activity *in vitro* [7,8], a number of clinical trials have investigated the combination of the two drugs using different schedules and dosages [9–15]. Most majority of these studies involved patients with advanced pancreatic carcinomas, and reported variable objective response rates, no effect on patient survival and a significant percentage of grade 3-4 haematological and gastroenteric toxicity and related mortality [9–15]. One of these studies in pancreatic carcinoma patients compared weekly GEM + 5-FU with GEM, finding no superiority of the combination in terms of the objective response rate, time to progression and overall survival [15]. As the two drugs act synergistically, along their activation pathways [7,8] and enhance their respective antitumour activity by interfering with pyrimidine synthesis and catabolism at different levels [7,8], we believe that the administration schedule may be extremely important in determining the toxicity and activity of the combination. We

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therefore investigated whether GEM affects 5-FU pharmacokinetics in advanced cancer patients.

2. Patients and methods

2.1. Eligibility criteria

The inclusion criteria required a histological diagnosis of malignancy, a performance status $\leqslant 2$ according to the Eastern Co-operative Oncology Group (ECOG) a life expectancy >3 months, normal renal and hepatic function, a white blood cell count $>2500\times10^6$/l, haemoglobin >90 g/l, a platelet cell count $>100\times10^9$/l, and normal cardiac function. The exclusion criteria were any major organ failure, central nervous system (CNS) involvement, second tumours and active infectious disease. The study was approved by our local (University) Ethics committee, and all of the patients gave their written informed consent.

2.2. Treatment

2.2.1. Experimental group

These patients received chemotherapy with GEM (1000 mg/m^2 in a 30-min intravenous (i.v.) on days 1, 8 and 16, plus folinic acid (FA) (100 mg/m^2) and 5-FU (400 mg/m^2) administered by a 30-min i.v. infusion on days 1–5 (1 h after GEM on day 1). The cycles were repeated every 4 weeks. The doses were extrapolated from previous studies of FUFA alone or in combinations with GEM.

2.2.2. Control group

All of these patients received FA (100 mg/m^2) and 5-FU (400 mg/m^2) administered by a 30-min i.v. infusion on days 1–5 every 4 weeks [20].

2.2.3. Pharmacokinetic evaluation

In order to evaluate the impact of GEM on 5-FU pharmacokinetics, 5-FU blood levels were monitored in two groups of patients receiving 5-FU and folinic acid (FUFA) (*control group*) or FUFA + GEM (*experimental group*). The venous blood samples (5 ml) were drawn and placed into heparinised tubes (Falcon) at 0, 5, 15, 30, 60 and 120 min after the beginning of 5-FU administration on days 1, 3 (in three patients) and 5 of the first, third and sixth cycles, and the plasma was immediately separated by centrifugation at 2000*g* for 10 min at 4 °C. The plasma samples were then transferred into polypropylene vials and frozen at −70 °C; they were thawed immediately before the assay.

High-performance liquid chromatography (HPLC) as described by Jung and colleagues [16] was used to determine the plasma concentrations of 5-FU, and its 5-fluorodeoxyuridine (5FdUrd) and 5-fluorouridine (5FUrd) metabolites after the samples had been deproteinised with 10% perchloric acid and filtered through 0.45-μm filters (Millipore). 5-FU and the metabolites were separated on a Hypersil ODS analytical column (4.6×150 mm; 5 μm particle size, Beckman Corp., USA) using methanol/acetic acid/water (3/0.05/96.95 v/v) as a mobile-phase at a flow rate of 0.8 ml/min, and assayed using a Waters HPLC system (Milford, MA, USA) with an ultraviolet (UV) detector set at 262 nm. The lower quantification limit for 5-FU, 5FUrd and 5FdUrd were all <23 ng/ml; the precision and accuracy rates evaluated as inter- and intraday variability were less than 10%. The area under the plasma concentration–time curve (AUC), elimination half-life ($t_{1/2}$), total plasma clearance (Cl_T), apparent distribution volume in the central compartment (V_D), and the maximum plasma concentration (C_{max}) of 5-FU were calculated for every patient using MK Model Software (Version 5, Biosoft, Cambridge, UK). As the short-term (30-min) infusions were considered as 5-FU bolus [17,18], the data of each course were fitted using a one-compartment model and linear elimination kinetics. The AUC was calculated from time zero to the last time point using the trapezoidal rule. Cl_T was determined by dividing the dose by AUC. The $t_{1/2}$ was calculated using a least-squares regression analysis of the data apparently belonging to the elimination phase. V_D in the central compartment was calculated as 5-FU dose/(elimination konstant (K_{el})×AUC). C_{max} was determined by visual inspection of the plasma concentration–time data [19].

2.2.4. Statistical analysis

Each experimental point was performed in triplicate. The statistical significance of all the differences between the mean values was determined using a two-tailed Student *t*-test. The Statistical Package for the Social Sciences (SPSS) (Version 6.0.1, 1994, SPSS Inc.) was used to analyse the pharmacokinetic data.

3. Results

3.1. Study design and patient characteristics

The study was designed to compare 5-FU pharmacokinetics in patients receiving the same FUFA treatment plus or minus GEM. All of the patients in both groups had metastatic gastroenteric carcinomas, and were treated in our Institution from November 1997 to January 1998.

The experimental group consisted of 20 patients (12 males and 8 females) with an average age of 66 years (range 34–82 years) belonging to a cohort of 51 individuals enrolled in a phase I-II clinical trial aimed at evaluating the toxicological and antitumour activity of GEM + FUFA in combination in various advanced gastroenteric carcinomas (the results have not yet been

fully published). They all had advanced stage disease and an ECOG performance status of $\leqslant 2$. 12 had pancreatic carcinomas, 5 had colorectal carcinomas, 2 had gallbladder carcinomas, and 1 a gastric carcinoma.

The control group consisted of 16 patients (9 males and 7 females) with a median age of 64 years (range 36–84 years) and an ECOG performance status of 0-2. They received FUFA chemotherapy for the standard treatment of various advanced stage disease gastroenteric carcinomas: 3 had pancreatic carcinomas, 5 had colorectal carcinomas, 6 had gallbladder carcinomas, and 2 had gastric carcinomas.

3.2. Pharmacokinetic analysis

The concentration–time curves of 5-FU in the patients in the two groups are shown in the Fig. 1. Analysis of the pharmacokinetic parameters showed that the patients in the experimental group (begun 1 h after the beginning of GEM administration) had a higher 5-FU AUC, C_{max}, and plasma $t_{2/2}$ values, and lower mean Cl_T and V_D (Table 1). None of the 5-FU pharmacokinetic parameter values in the individual patients changed significantly between day 1 and day 3 or day 5 of the first cycle (data not shown), thus suggesting that effect of GEM on 5-FU pharmacokinetics is a long-term effect and lasts longer than 5 days. Furthermore, there was no difference in the individual 5-FU pharmacokinetic parameter values between the first, third and sixth cycles (data not shown), suggesting that the effect of GEM on 5-FU pharmacokinetics is reproducible and not lost during the treatment. The 5-FU cytotoxic metabolite precursors, 5FdUrd and 5FUrd, were only detectable in the patients in the experimental group (15 and 16 patients, respectively). The two nucleoside metabolites did not interfere with the GEM and GEM metabolite readings; in fact, in our assay, 1 h after the beginning of the GEM administration, when the 5-FU pharmacokinetic monitoring started, the GEM metabolites were no longer detectable in the plasma (data not shown), thus excluding the possibility of an intra-assay interference.

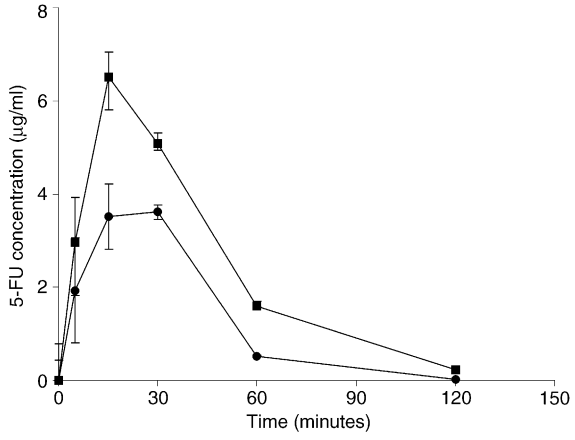

Fig. 1. 5-Fluorouracil (5-FU) (μg/ml) concentration–time curves + - standard error in 20 patients receiving difluoro-2′,2′-deoxycytidine or gemcitabline (GEM) plus 5-fluorouracil and folinic acid (FUFA) (■) and a control group of 16 patients receiving FUFA alone (at the same dosage and schedule) (●).

Table 1

PK parameters	GEM + FUFA	FUFA	Statistical significance
AUC (μg/ml/min)	555.2 ± 208.67	244 ± 89.14	$P < 0.001$
C_{max} (μg/ml)	6.6 ± 3.00	4.5 ± 2.34	$P < 0.02$
plasma $t_{\frac{1}{2}}$ (mins)	22.2 ± 10.35	14.5 ± 5.34	$P < 0.05$
Cl_T (ml/min/m^2)	903.6 ± 429.05	1946.0 ± 896.72	$P < 0.001$
V_D (l)	29.7 ± 24.18	40.2 ± 18.59	$P < 0.001$

AUC, area under the plasma concentration time curve; C_{max}, maximum plasma concentration; Cl_T, total plasma clearance; $t_{\frac{1}{2}}$, elimination half-life; V_D, apparent distribution volume in the central compartment; FUFA, 5-fluorouracil (5-FU) and folinic acid; GEM, gemcitabine; Pk, Pharmacokinetic.

4. Discussion

Our pharmacokinetic results suggest that GEM enhances systemic exposure to 5-FU in cancer patients. The 5-FU AUC, and its elimination half-life ($t_{1/2}$) were significantly higher in the experimental group receiving GEM + FUFA than in the control group receiving FUFA alone.

The two metabolite precursors, 5FUrd, and 5FdUrd, were only observed in the experimental group. As these metabolites are diffusible across the membrane, and their concentrations are in equilibrium with the extracellular compartment, their enhanced serum concentration is assumed to indicate a higher intracellular production. This observation may be of interest because the two intracellular 5-FU cytotoxic metabolites, 5FdUMP and 5FUTP, are the final products of the subsequent phosphorylations of 5FdUrd and 5FUrd, respectively. The results of most clinical trials testing the combination of the two drugs suggest that the GEM plus 5-FU (±FA) administration given by short or prolonged i.v. infusion has significant anti-tumour activity in patients with pancreatic carcinoma and other gastroenteric cancers, including colorectal carcinoma [7–15]. One of the largest of these study, the E2297 phase III trial, by the ECOG, compared a weekly schedule of GEM (1 g/m^2) plus FU (600 mg/m^2) with GEM alone in patients with pancreatic carcinoma and reported very disappointing results: the combination, in fact showed no advantage in terms of overall survival (6.7 versus 5.4 months), progression-free survival (3.4 versus 2.2 months) or the response rate (5.6% versus 6.9%) and was burdened by a higher degree of therapy-related

toxicity [15]. We have recently concluded the first phase I-II clinical trial testing GEM + 5-FU and FA in patients with advanced cancers (including pancreatic, colorectal and biliary tract carcinomas), which was designed on the basis of previous *in vitro* studies demonstrating synergistic antitumour activity and positive pharmacological interactions between GEM and 5-FU (and FA) in colon carcinoma cell lines. These studies showed that the two drugs have synergistic antitumour activity and induce apoptosis when GEM is given before 5-FU [22]. As mentioned in the Results section, the patients in the experimental group of the present study belonged to a cohort of 51 patients enrolled in the above described phase I-II trial. In that study, 8 patients were not evaluable for response because of a premature treatment discontinuation due to side-effects (6 patients) or sudden life-threatening tumour-related complications (2 patients) during the first cycle of treatment cycle. A demonstrable clinical response was observed in eleven patients (8/36 with pancreatic carcinoma, 1/8 with colon carcinoma, and 2/5 with biliary tract carcinoma) and there were 19 disease stabilisation (17 pancreatic carcinomas, one colon carcinoma and one unknown primary site carcinoma), some of which were not responsive to 5-FU or GEM as single agents [21]. The study was unfortunately hampered by the occurrence of 5 cases of grade 4 gastroenteric toxicity, 3e of which are reported in the present study, with two toxic deaths; an observation that leads of to believe that GEM can effectively enhance the antitumour activity of FUFA, but also increases the toxicity in cancer patients. The synergy of the two drugs is probably due to intracellular interactions along their respective biochemical pathways of activation and metabolism; however, our pharmacokinetic findings may partially explain the clinical effects observed when GEM and 5-FU are given in combination. It has been previously shown that there is a very close relationship between changes in plasma 5-FU pharmacokinetic parameters and its antitumour activity and toxicity in cancer patients [22], and so the enhanced 5-FU systemic exposure found in the presence of GEM may well explain the higher level of toxicity and antitumour activity observed in this clinical trial.

We do not yet have data to explain the mechanism causing the occurrence of such events, but it is very likely that $t_{1/2}$, Cl_T, AUC, C_{max} and V_D alterations are due to GEM or GEM metabolite interactions in 5-FU metabolism and elimination (e.g. 5-FU re-uptake by the renal tubules). Different administration schedules may therefore explain the different results of studies testing the two drugs in combination, and also partially explain the failure of the E2297 phase III trial.

Our pharmacological results should be taken into account when designing clinical trials testing GEM and 5-FU in combination in order to maximise the antitumour effects and reduce the occurrence of side-effects.

References

1. Chu E, Mota AC, Fogarasi MC. Antimetabolites. In De Vita V, Hellman S, Rosenberg SA, eds. *Cancer Principles and Practice of Oncology*, 6th ed. Philadelphia, Lippincott Williams and Wilkins, 2001, 388–415.
2. Grem JL. 5-Fluoropyrimidines. In Chabner BA, Longo DL, eds. *Cancer Chemotherapy and Biotherapy: Principles and Practice*, 2nd ed. Philadelphia, Lippincot William & Wilkins, 1996, 149–163.
3. Grem JL, Takimoto CH, Multani P, *et al*. Antimetabolites. *Cancer Chemother Biol Response Modif* 1999, **18**, 1–38.
4. Plunkett W, Huang P, Searcy CE, Gandhi V. Gemcitabine: preclinical pharmacology and mechanisms of action. *Semin Oncol* 1996, **23**(Suppl. 10), 3–15.
5. Burris 3rd HA, Moore M, Andersen J, *et al*. Improvements in survival and clinical benefit with gemcitabine as first line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997, **15**, 2403–2413.
6. Ulrich-Pur H, Kornek GV, Raderer M, *et al*. A phase II trial of bi-weekly high dose gemcitabine for patients with metastatic pancreatic adenocarcinoma. *Cancer* 2000, **88**, 2505–2511.
7. Peters GJ, van der Wilt CL, van Moorsel CJ, Kroep JR, Bergman AM, Ackland SP. Basis for effective combination cancer chemotherapy with antimetabolites. *Pharmacol Ther* 2000, **87**, 227–253.
8. Schulz L, Schalhorn A, Wilmanns W, Heinemann V. Synergistic interaction of dFdC and 5-FU in colon cancer cells. *Proc Am Soc Clin Oncol* 1998, **17**, 251a.
9. Berlin JD, Alberti DB, Arzoomanian RZ, et al., A phase I study of gemcitabine, 5-fluorouracil and leucovorin in patients with advanced, recurrent, and/or metastatic solid tumors, Invest. New Drugs, 16, 1998–1999, 325–330.
10. Hidalgo M, Paz-Ares L, Hitt R, Cortes-Funes H. Phase I-II study of gemcitabine combined with continuous infusion 5-fluorouracil as first-line chemotherapy in locally advanced and metastatic pancreatic cancer. *Proc Am Soc Clin Oncol* 1997, **16**, 290a.
11. Hidalgo M, Castellano D, Paz-Ares L, *et al*. Phase I-II study of gemcitabine and fluorouracil as a continuous infusion in patients with pancreatic cancer. *J Clin Oncol* 1999, **17**, 585–592.
12. Cascinu S, Silva RR, Barni S, *et al*. A combination of gemcitabine and 5-fluorouracil in advanced pancreatic cancer, a report from the Italian Group for the Study of Digestive Tract Cancer (GISCAD). *Br J Cancer* 1999, **80**, 1595–1598.
13. Madajewicz S, Hentschel P, Burns P, *et al*. Phase I chemotherapy study of biochemical modulation of folinic acid and fluorouracil by gemcitabine in patients with solid tumor malignancies. *J Clin Oncol* 2000, **18**, 3553–3557.
14. Oettle H, Riess H. Gemcitabine in combination with 5-fluorouracil with or without folinic acid in the treatment of pancreatic cancer. *Cancer* 2002, **95**, 912–922.
15. Berlin JD, Catalano P, Thomas JP, Kugler JW, Haller DG, Benson AB. Phase III study of gemcitabine in combination with fluorouracil versus gemcitabine alone in patients with advanced pancreatic carcinoma: Eastern Cooperative Oncology Group Trial E2297. *J Clin Oncol* 2002, **20**, 3270–3275.
16. Jung M, Berger G, Pohlen U, Pauser S, Reszka R, Buhr HJ. Simultaneous determination of 5-fluorouracil and its active metabolites in serum and tissue by high-performance liquid chromatography. *J Chromatogr B Biomed Sci Appl* 1997, **702**, 193–202.
17. Joulia JM, Pinguet F, Ychou M, *et al*. Pharmacokinetics of 5-Fluorouracil (5-FUra) in patients with metastatic colorectal cancer receiving 5-FUra bolus plus continuous infusion with high dose folinic acid (LV5FU2). *Anticancer Res* 1997, **17**, 2727–2734.

18. Wattanatorn W, McLeod HL, Macklon F, Reid M, Kendle KE, Cassidy J. Comparison of 5-Fluorouracil pharmacokinetics in whole blood, plasma, and red blood cells in patients with colorectal cancer. *Pharmacoteraphy* 1997, **17**, 881–886.
19. Rowland M, Thomas NT. *Clinical Pharmacokinetics, Concepts and Application*. Philadelphia, William and Zilkins, 1996.
20. Machover D, Goldschmidt E, Chollet P, *et al*. Treatment of advanced colorectal and gastric adenocarcinoma with 5-fluorouracil and high-dose folinic acid. *J Clin Oncol* 1986, **4**, 685–689.
21. Correale, P., Cerretani, D., Petrioli, R., Marsili, S., Giorgi, G. and Francini, G. Gemcitabine (dFdC) and 5 fluorouracil (5FU) in the treatment of patients with pancreas and colo-rectal carcinoma: a clinical and pharmacological study. *ICACT 10th International Congress on Anti-Cancer Treatment*, 31 January–3 February 2000, Paris, France, Abstr. I-293.
22. Milano G, Etienne MC, Renee N, *et al*. Relationship between fluorouracil systemic exposure and tumor response and patient survival. *J Clin Oncol* 1994, **12**, 1291–1295.