Eur J Cancer, Vol. 29A, No. 13, pp. 1851–1854, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00

Circadian Rhythm-modulated Chemotherapy with High-dose 5-Fluorouracil: a Pilot Study in Patients with Pancreatic Adenocarcinoma

F. Bertheault-Cvitkovic, F. Lévi, S. Soussan, S. Brienza, R. Adam, M. Itzhaki, J.L. Misset and H. Bismuth

From November 1986 to April 1989, 16 patients with advanced measurable pancreatic carcinoma were involved in a pilot phase I–II study. 5-Fluorouracil was given every 3 weeks by 5-day continuous chronomodulated venous infusion (CMVI) with peak 5-FU delivery at 4 a.m. Intrapatient dose escalation started at 1200 mg/m²/day up to 1600 mg/m²/day in the absence of grade III (WHO) toxicity. Mucositis and diarrhoea were dose limiting in the 131 cycles given. Three partial responses (21%) and 5 stable diseases were seen in the 14 patients with measurable disease. Dose intensity after three or after six courses (1800 mg/m²/week) was significantly correlated with time to progression (Pearson $r = 0.64$; $P < 0.004$). These results, although modest, support a multicentre phase II trial with 5-FU CMVI.
Eur J Cancer, Vol. 29A, No. 13, pp. 1851–1854, 1993.

INTRODUCTION

5-FLUOROURACIL (5-FU) has remained the reference drug against metastatic pancreatic cancer despite its rather modest antitumour activity. Thus, response rates ranged from 10 to 15% in randomised trials, with a 95% upper confidence limit barely exceeding 20%. Other drugs such as mitomycin-C, streptozotocin or ifosphamide resulted in even lower antitumour activity [1].

Combination chemotherapy did not improve response or survival rates as compared to single agents [2], despite earlier encouraging results [3]. Reported median survival usually ranged between 2 and 7 months, whatever the chemotherapy regimen [1–4]. Nonetheless, the association of 5-FU with radiation therapy might increase survival of patients with locally advanced disease [5].

5-FU has commonly been administered as a 5-day continuous venous infusion at a constant rate. Daily doses have usually ranged from 0.8 to 1.2 g/m², each course (4–6 g/m²) being repeated every 3 to 4 weeks. When 5-FU was administered at a constant rate for 5 or 14 days, its plasma levels predictably doubled along the 24-h time scale [6, 7]. The circadian rhythm in the activity of dehydropyrimidine dehydrogenase (DPD), an enzyme which catabolises 5-FU [7–10], and/or the circadian rhythm in hepatic blood flow [11] may account for these predictable changes in 5-FU pharmacokinetics. Whatever the underlying mechanisms, 5-FU was shown to be least toxic in mice when they were dosed in the light (rest) span, at a time of high liver DPD activity [12, 13]. Nonetheless, dosing 5-FU at a time when it was less toxic was associated with an increased antitumour activity against two transplanted murine colon carcinomas [14].

Mechanisms of cellular resistance (and susceptibility) to 5-FU are multifactorial and may differ further between normal and cancer cells [15–17]. In the present trial, we have tentatively extrapolated the time of least toxicity of 5-FU in cancer patients from murine data, by referring it to the sleep–wakefulness cycle of either species. Thus, DPD activity was highest at night in human subjects and during daytime in mice or rats [7–10]; the reverse was true for bone marrow proliferation in these species [18–20]. Our goal was to take advantage of a decrease in 5-FU toxicity brought about by chronomodulation of drug delivery, for increasing dose intensity and attempting to improve tumour control.

A similar investigation had been performed in patient suffering from colorectal metastatic cancer and led to an apparent doubling of the antitumour efficacy of chemotherapy [21].

PATIENTS AND METHODS

From November 1986 to April 1989, 16 patients with progressive metastatic or locally advanced pancreatic cancer were registered in this trial. They gave their oral informed consent. No ethics committee existed at the time when this trial was conducted. Patients' characteristics are summarised in Table 1. Of 6 patients who had prior cephalic duodenopancreatectomy, 2 patients had no assessable disease, but were evaluated for toxicity. 6 patients had locoregional disease, one had metastatic disease and the last 7 evaluable patients had both metastatic and locoregional targets.

5-FU was given as a 5-day continuous venous infusion with a chronomodulated drug delivery rate, using a programmable-in-time ambulatory pump (Chronopump, Autosyringe, U.S.A.). Peak delivery was scheduled at 4 a.m. (3.7 ml/h) and no delivery ('keep vein open' rate) was programmed from 6 p.m. to 10 p.m. (Fig. 1). The 60 ml syringe (Beckton-Dickinson) was changed daily at 6 p.m. and the pump was started at this time. In a previous study in patients with metastatic colorectal cancer, 203

Correspondence to F. Bertheault-Cvitkovic at the Centre de Chronothérapie.
F. Bertheault-Cvitkovic, F. Levi, S. Soussan, S. Brienza, M. Itzhaki and J.L. Misset are at the Centre de Chronothérapie, Service des Maladies Sanguines, Immunitaires et Tumorales; F. Bertheault-Cvitkovic, F. Levi, M. Itzhaki and J.L. Misset are also at the Laboratoire 'Rythmes Biologiques et Chronothérapeutique' (Université Paris XI), Institut du Cancer et d'Immunogénétique; and R. Adam and H. Bismuth are at the Service de Chirurgie Hépatobiliaire, Hôpital Paul Brousse, 94800 Villejuif, France.
Revised 11 Mar. 1993; accepted 8 Apr. 1993.

Table 1. Patients' characteristics

	No. of patients	
Male:female	12:4	
Median age, years (range)	56 (40–82)	
Peformance status (WHO)		
0	2	
1	3	
2	5	
3	6	
Number of measurable sites		
0	2	
1	2	
2	6	14
3 or more	6	
Sites involved		
Pancreas		
Head	9	13
Body or tail	4	
Regional lymph nodes	9	
Liver		
< 50%	4	8
≥ 50%	4	
Epiduritis	1	
Previous treatment on progression		
Somatostatin analogue	2	
5-FU–folinic acid	3	
Other chemotherapy	2	
Interval from diagnosis to protocol, months (range)	4 (2–22)	
Histology		
Adenocarcinoma	15	
Ampullar carcinoma	1	
CA19-9 > 40 U/ml	15	
median, U/ml (range)	324 (24–16540)	

courses had been administered with this device. Among 1015 patient-days, 47 technical problems occurred of which only seven were related to the chronopump (three programming errors, four low battery alarms).

The appropriate daily dose of 5-FU was dissolved in 5% dextrose if needed, so that its concentration ranged from 25 to 50 mg/ml. Thus, the daily volume which was infused was always 50 ml. The syringe was connected to an implanted venous access port (Port-a-Cath™, Pharmacia) via a 1.50 m long catheter

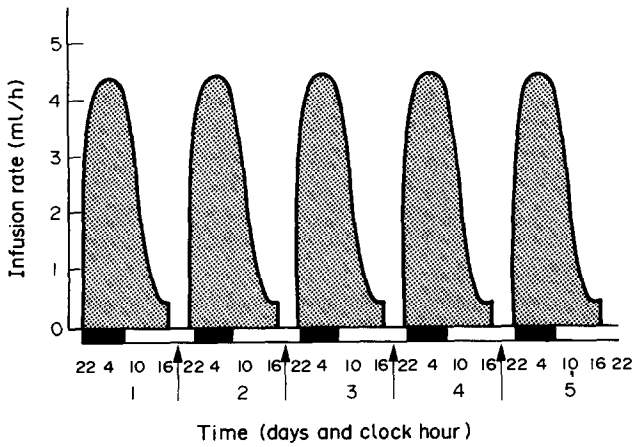

Fig. 1. Scheme of 5-FU delivery from day 1 to day 5. Arrows indicate daily syringe changes.

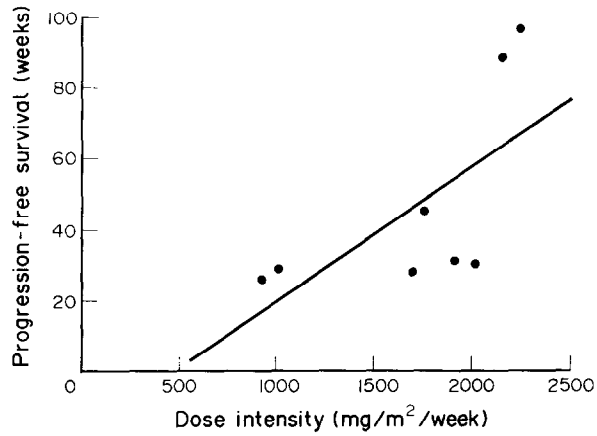

Fig. 2. Correlation between dose intensity of 5-FU over initial three courses and progression-free survival.

(internal volume 2 ml), which was filled with the same solution of 5-FU at the beginning of each course.

Daily 5-FU dose was 1200 mg/m² in the first course for each patient, then escalated up to 1600 mg/m² in the absence of any WHO grade II toxicity or greater in 200 mg/m² steps. In case of grade II toxicity, the dose administered remained the same in the subsequent course. If toxicity was grade III or greater, daily dose was reduced in 200 mg/m² steps. Each course was repeated following a 16-day treatment-free interval. Treatment was given until disease progression. All patients were treated as out-patients.

The received dose intensity (DI) was calculated over the first three courses and over the first six courses, as recommended by the NCI [22] using the following formula: total dose of 5-FU given over three (or six) courses (mg/m²) : (total number of days of therapy : 7).

Computed tomography of the abdomen and/or thorax and echography was performed every three to four courses with markers as corroborating evidence. Objective responses, as defined with standard criteria, were reviewed by the same radiologist.

RESULTS

One hundred and thirty-one courses were administered, with a median number of six courses per patient (range two to 27). All patients were ambulatory. Pump reliability was excellent and was similar to our previous experience [23].

Toxicity was acceptable; no grade IV occurred. Mucositis and diarrhoea were dose limiting. Grade III mucositis was observed in seven courses (5%) in 6 patients (37%). Grade III diarrhoea was encountered in two courses (2%) in 2 patients (12%). No grade III or greater toxicity was found with regard to haematology, skin, hair, central nervous system, heart or peripheral nerves. No angina pectoris was observed (Table 2).

Good compliance with protocol treatment was observed; median total given per patient was 34.1 g/m² (13–170.5 g/m²). Median DI was 1800 mg/m²/week for the first three courses in 13 assessable patients and 1740 mg/m²/week for the first six courses in 9 assessable patients (range 1030–2350).

3 of the 14 patients with measurable disease achieved an objective response (21%). 5 patients had stable disease (SD) and disease progressed (PD) in 6 patients. Partial responses (PR) were observed in liver (2 patients) or in pancreas (1 patient). 1 patient with PR had previously progressed while receiving 5-FU

Table 2. Toxicity

	Incidence of toxicity by course (n = 131) WHO grade				Maximal toxicity per patient (n = 16) WHO grade			
	1	2	3	4	1	2	3	4
Diarrhoea	19* (14.5)	9 (6.9)	2 (1.5)	0	2† (12.5)	4 (25)	2 (12.5)	0
Mucositis	26 (19.8)	12 (9.2)	7 (5.3)	0	4 (25)	3 (18.7)	6 (37.5)	0
Nausea, vomiting	12 (9.2)	2	1	0	3 (18.7)	2 (12.5)	1 (6.2)	0
Alopecia	2 (1.5)	0	0	0	2 (12.5)	0	0	0
Dermatitis	15 (11.4)	4 (3)	0	0	3 (18.7)	2 (12.5)	0	0
Others	0	0	0	0	0	0	0	0

*Number of courses (percentage). †Number of patients (percentage).

and folinic acid (5-day schedule). In every case (PR, SD, PD) the time course of serum carcinoembryonic antigen (CEA) and/or CA 19.9 correlated with clinical response (increase in case of PD, stabilisation or minor decrease in case of SD and reduction by more than 50% in case of PR).

Median time to progression and median survival were, respectively, 30 weeks (range 24–96) and 43 weeks (range 8–108). Survival of responders was 41, 45+ (lost to follow-up) and 52 weeks. Survival in the 5 patients with stable disease was 26, 31+, 96, 96 and 108 weeks. This long survival was apparently unrelated to a long natural history of pancreatic cancer in those patients, since time from diagnosis to treatment was less than 4 months in the 3 patients with PR and only 1 of the 5 patients with stable disease had a long time interval from diagnosis to treatment (t d t) (88 weeks of t d t and 108 weeks of survival).

A statistically significant correlation was observed between DI, response rate and time to progression (TTP). All 3 PR patients and 3 of the SD patients had received a DI of 1500 mg/m^2/week or greater over the first six courses of therapy. A strong correlation was found between DI over three of six courses of therapy. A strong correlation was found between DI over three or six courses and TTP (Fig. 2) (non-parametric Spearman test: $r = 0.881$, $P = 0.004$ and $r = 0.883$, $P = 0.01$, respectively). No such correlation was found between DI and survival, as a result of the prolonged survival of a single previously extensively pretreated patient with very slowly progressing disease.

DISCUSSION

This pilot study suggests a role for 5-FU DI on antitumour efficacy in advanced pancreatic cancer as is the case for metastatic colorectal cancer [24, 25]. Such high dose intensities were obtained without excessive toxicity through a 24-h chronomodulation of drug delivery. Similar findings have been achieved for doxorubicin, floxuridine and oxaliplatin in randomised phase I comparisons of flat vs. chronomodulated delivery [26–28].

The present results may be accounted for by the delivery of higher doses of 5-FU at a time when healthy tissues catabolise it faster and/or transform it more slowly into its active cytotoxic forms, fluorouridine monophosphate (FUMP) and fluorodeoxyuridine monophosphate (FdUMP) [7–10, 29–31]. Nonetheless, a possible alteration of these rhythms by the present schedule deserves to be investigated, both in healthy tissues and in tumours. We believe such exploration should be part of a larger phase II trial of circadian rhythm-modulated delivery of 5-FU in patients with advanced pancreatic cancer. This might help to select those long-term survivors who benefitted from this mode of therapy.

1. Warshaw AL, Del Castillo CF. Pancreatic carcinoma. *New Engl J Med* 1992, **326**, 455–465.
2. Cullinam SA, Moertel CG, Wieand HS, *et al.* A phase III trial on the therapy of advanced pancreatic carcinoma. *Cancer* 1990, **65**, 2207–2212.
3. Mallinson CN, Rake MO, Cocking JB. Chemotherapy in pancreatic cancer: results of a controlled, prospective, randomized, multicentre trial. *Br Med J* 1980, **281**, 1589–1591.
4. Wils JA. Current status of chemotherapy in metastatic pancreatic cancer. *Anticancer Res* 1989, **9**, 1027–1032.
5. Wagener DJT, Van Hoesel QGCM, Yap SH, *et al.* Phase III trial of 5-fluorouracil, adriamycin and cisplatin (FAP) followed by radiation and 5-fluorouracil in locally advanced pancreatic cancer. *Cancer Chemother Pharmacol* 1989, **25**, 131–134.
6. Petit E, Milano G, Lévi F, Thyss A, Bailleul F, Schneider M. Circadian rhythm-varying plasma concentration of 5-fluorouracil during a five-day continuous venous infusion at a constant rate in cancer patients. *Cancer Res* 1988, **48**, 1676–1679.
7. Harris B, Song R, He Y, *et al.* Circadian rhythm of rat liver dihydropyrimidine dehydrogenase : possible relevance to fluoropyrimidine chemotherapy. *Biochem Pharmacol* 1988, **37**, 4759–4762.
8. Harris B, Song R, Soong S, Diasio RB. Circadian variation of 5-fluorouracil catabolism in isolated perfused rat liver. *Cancer Res* 1989, **49**, 6610–6614.
9. Tuchman M, Roemeling RV, Hrushesky WJM, O'Dea RF. Dihydropyrimidine dehydrogenase activity in human blood mononuclear cells. *Enzyme* 1989, **42**, 15–24.
10. Harris B, Song R, Soong S, Diasio RB. Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels : evidence for circadian variation of plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. *Cancer Res* 1990, **50**, 197–201.
11. Lemmer B, Nold G. Circadian changes in estimated hepatic blood flow in healthy subjects. *Br J Clin Pharmacol* 1991, **32**, 627–629.
12. Burns ER, Beland SS. Effect of biological time on the determination of the LD_{50} of 5-fluorouracil in mice. *Pharmacology* 1984, **28**, 296–300.
13. Mormont MC, Boughattas N, Lévi F. Mechanisms of circadian rhythms in the toxicity and the efficacy of anticancer drugs : relevance for the development of new analogs. In Lemmer B, ed. *Chronopharmacology : Cellular and Biochemical Interactions*. New York, Marcel Dekker, 1989, 395–437.
14. Peters GJ, Van Dijk J, Nadal JC, Van Groeningen CS, Lankelma J, Pinedo HM. Diurnal variation in the therapeutic efficacy of 5-fluorouracil against murine colon cancer. *In Vivo* 1987, **1**, 113–118.

15. Naguib FNM, El Kouni MH, Cha S. Enzymes of uracil catabolism in normal and neoplastic human tissues. *Cancer Res* 1985, **45**, 5405–5412.
16. Kovach JS, Beart RW Jr, Svingen P, *et al.* Concentration of 5-fluorouracil (FU) and metabolites in human colon carcinomas and normal tissues after i.v. FU. *Proc Am Soc Clin Oncol* 1986, **5**, 177.
17. Aiba K, Allegra CJ, Park JG, Drake JC, Chabner BA. An investigation of the determinants of 5-fluorouracil (FU) resistance in human colon carcinoma cell lines. *Proc Am Assoc Cancer Res* 1989, **30**, 2383.
18. Lévi F, Blazsek I, Ferlé-Vidovic A. Circadian and seasonal rhythms in murine bone marrow colony-forming cells affect tolerance for the anticancer agent 4'-0-tetrahydropyranyladriamycin (THP). *Exp Hematol* 1988, **16**, 696–701.
19. Smaaland R, Laerum OD, Lote K, Sletvold O, Sothern RB, Bjerknes R. DNA synthesis in human bone marrow is circadian stage dependent. *Blood* 1991, **77**, 2603–2611.
20. Smaaland R, Laerum OD, Sothern RB, Sletvold O, Bjerknes R, Lote K. Colony-forming unit-granulocyte-macrophage and DNA synthesis of human bone marrow on circadian stage-dependent and show covariation. *Blood* 1992, **79**, 2281–2287.
21. Lévi F, Misset JL, Brienza S, *et al.* A chronopharmacologic phase II clinical trial with 5-FU, folinic acid and oxaliplatin using an ambulatory multichannel programmable pump. *Cancer* 1992, **69**, 893–900.
22. Longo DL, Duffey PL, De Vita VT, *et al.* The calculation of actual or received dose intensity: a comparison of published methods. *J Clin Oncol* 1991, **9**, 2042–2051.
23. Soussan A. Chronothérapie des cancers colorectaux par perfusion veineuse continue sur 5 jours de 5-fluorouracile (5-FUra) administré par pompe programmable. *Mémoire en vue du Diplôme d'Etudes et de Recherches en Biologie Humaine*, Université Paris XI, 1988.
24. Shah A, MacDonald W, Goldie J, Gudanskas G, Brisebois B. 5-FU infusion in advanced colorectal cancer : a comparison of three dose schedules. *Cancer Treat Rep* 1985, **69**, 739–742.
25. Hryniuk W, Figueredo A, Goodyear M. Applications of dose-intensity to problems in chemotherapy of breast and colorectal cancer. *Semin Oncol* 1987, **14**, 3–11.
26. Bailleul F, Lévi F, Metzger G, Regensberg C, Reinberg A, Mathé G. Chronotherapy of advanced breast cancer with continuous doxorubicin infusion via an implantable programmable device. *Proc AACR* 1987, **28**, 771.
27. Von Roemeling R, Hrushesky W. Circadian patterning of continuous floxouridine infusion reduces toxicity and allows higher dose intensity in patients with widespread cancer. *J Clin Oncol* 1989, **7**, 1710–1719.
28. Caussanel JP, Lévi F, Brienza S, *et al.* Phase I trial of 5-day continuous venous infusion of oxaliplatinum at circadian-modulated vs constant rate. *J Natl Cancer Inst* 1990, **82**, 1046–1050.
29. El Kouni M, Naguib F. Circadian rhythm of hepatic pyrimidine metabolizing enzymes and plasma uridine concentration in mice. *Proc 5th Int Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–2.
30. Zhang R, Lu E, Liu T, *et al.* Circadian rhythm of rat spleen cytoplasmic thymidine kinase: possible relevance to 5-fluorodeoxyuridine chemotherapy. *Proc 5th Int Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–3.
31. Diasio R, Zhang R, Lu Z, *et al.* Circadian variation of fluoropyrimidine catabolic enzymes:importance to fluorodeoxyuridine (FdURD) chemotherapy. *Proc 5th Intern Conf Chronopharmacol.* Florida, Amelia Island, 1992, IV–1.

Acknowledgements—We are indebted to J.P. Caussanel and G. Metzger for their help at various stages of this study and to M. Levi for excellent editorial assistance.

Eur J Cancer, Vol. 29A, No. 13, pp. 1854–1857, 1993.
Printed in Great Britain

0959–8049/93 $6.00 + 0.00
Pergamon Press Ltd

A Toxic Interaction Between Mitomycin C and Tamoxifen Causing the Haemolytic Uraemic Syndrome

A. Montes, T.J. Powles, M.E.R. O'Brien, S.E. Ashley, J. Luckit and J. Treleaven

A comparison of patients receiving combination chemotherapy with mitomycin C, mitozantrone and methotrexate (3M) with and without tamoxifen for treatment of primary breast cancer indicates an increased risk of anaemia ($P < 0.0001$) and thrombocytopenia ($P < 0.001$), but not leucopenia for patients receiving tamoxifen with their chemotherapy compared to those receiving the chemotherapy alone. Furthermore, 9 out of 94 patients receiving tamoxifen with 3M developed progressive anaemia, thrombocytopenia and abnormal renal function as early features of microangiopathic haemolytic anaemia, progressing on to various degrees of the haemolytic uraemic syndrome (HUS). This is only rarely seen with patients receiving mitomycin C alone at higher doses than used in the 3M combination and in the presence of active metastatic disease. This syndrome can be fatal and 1 of our 9 patients died. These observations indicate that there may be an interaction between tamoxifen and mitomycin C, causing an increased incidence of anaemia, thrombocytopenia and an increased risk of HUS. The combination of these two drugs should be avoided or carefully monitored.
Eur J Cancer, Vol. 29A, No. 13, pp. 1854–1857, 1993.

INTRODUCTION

THE HAEMOLYTIC uraemic syndrome (HUS) is an acquired syndrome consisting of intravascular haemolysis, thrombocytopenia (TCP) and acute renal failure with hypertension, neurological symptoms, pulmonary oedema and intolerance to blood transfusions. It was originally described by Gasser and his co-workers in 1955 as a fatal illness affecting five small children [1]. It seems probable that the major cause of the syndrome is localised microangiopathy mainly in the glomerular capillaries, this being primarily produced by an abnormal interaction between platelets and the vascular endothelium, probably mediated by abnormalities by prostacyclin production which