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A phase I and pharmacokinetic study of gemcitabine given by 24-h hepatic arterial infusion

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ABSTRACT

Purpose: This study was performed to assess the toxicities, the maximum-tolerated dose (MTD), the pharmacokinetics and the anti-tumour activity of gemcitabine given by 24-h hepatic arterial infusion (HAI).

Patients and methods: Patients with liver malignancies received gemcitabine by 24-h HAI, weekly × 3, every 4 weeks. On day 1 or day 8 of the first cycle, patients received one administration by 24-h intravenous infusion for pharmacokinetic comparison and to determine hepatic extraction.

Results: Thirteen patients received gemcitabine at the dose levels of 75, 135 and 180 mg/m^2. The MTD was 180 mg/m^2 with thrombocytopaenia as the dose-limiting toxicity. Pharmacokinetic analysis showed a significantly lower maximum gemcitabine plasma concentration (C_{max}: HAI, 26, 80 and 128 nM, respectively; IV, 229, 264 and 293 nM, respectively) and area under the plasma-concentration-versus-time curve (AUC_{0-24h}: HAI, 386, 1247 and 2033 nmol × h/L, respectively; IV, 3526, 4818 and 5363 nmol × h/L, respectively) during HAI, compared with intravenous infusion (both $P < 0.001$). Additionally, the mean hepatic extraction ratios of gemcitabine at the 75, 135 and 180 mg/m^2 dose level were 0.89, 0.75 and 0.55, respectively. Hepatic extraction decreased linearly with increasing dose. The C_{max} and AUC_{0-24h} of 2′,2′-difluoro-2′-deoxyuridine, the deaminated product of gemcitabine, were similar for HAI and intravenous infusion. Seven patients had stable disease for a median duration of 9 months (range: 2–11 months).

Conclusions: Gemcitabine given by 24-h HAI was well tolerated and resulted in significantly lower systemic gemcitabine plasma concentrations than intravenous infusion due to a relatively high hepatic extraction.

1. Introduction

The administration of chemotherapy by hepatic arterial infusion (HAI) offers potential advantages over intravenous therapy for the treatment of patients with tumours confined to the liver. HAI results in increased local drug concentrations. Furthermore, if the drug is eliminated by hepatic extraction, the hepatic arterial administration will yield lower systemic

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concentrations and hence less toxicity may be expected than by intravenous (i.v.) administration.[1–3] Since liver malignancies derive their blood supply mainly from the hepatic artery, while normal liver tissue is supplied mostly by the portal vein high drug levels will especially be achieved at the site of the malignant disease.[4]

HAI has been studied most extensively in patients with liver metastases of colorectal cancer using fluorodeoxyuridine (FUDR) or 5-fluorouracil (5-FU). Despite the significantly higher response rates for HAI than intravenous infusion, most of these studies did not report a significant prolongation of survival, even when meta-analyses were performed.[5,6] Further investigation of the possibilities of HAI continues and also includes the study of new agents.

Gemcitabine (2′,2′-difluorodeoxycytidine) is a deoxycytidine analogue with activity against various malignancies.[7–10] Gemcitabine has to be phosphorylated intracellularly to the active forms: gemcitabine diphosphate and triphosphate.[11] The rate-limiting enzyme in this process is deoxycytidine kinase (dCK), and its activity has been demonstrated to be related with the response of experimental tumours.[12] The main cytotoxic effect of gemcitabine involves the competitive incorporation of gemcitabine triphosphate (dFdCTP) with deoxycytidine triphosphate (dCTP) into replicating DNA. Once dFdCTP is incorporated, DNA polymerase is able to add only one more nucleotide (masked chain termination) after which the DNA synthesis is inhibited and cell death occurs. Gemcitabine diphosphate (dFdCDP) induces a self-potentiating mechanism by inhibiting ribonucleotide reductase, thereby reducing the amount of intracellular dCTP. Deamination of gemcitabine occurs rapidly and extensively by the enzyme deoxycytidine deaminase (dCDA) to the inactive metabolite 2′,2′-difluoro-2′-deoxyuridine (dFdU).[11] Thus, dCK and dCDA are two important enzymes in the activation and inactivation of gemcitabine, respectively. Increased dCK levels have been observed in human cells of various malignancies.[13,14] This could give an advantage for liver malignancies compared to normal liver tissue in selectively activating the gemcitabine delivered by HAI. Also, since the liver has a high dCDA activity,[15,16] a high hepatic extraction of gemcitabine can be expected. Gemcitabine is therefore theoretically a suitable drug for HAI. In two other studies HAI of gemcitabine was investigated, both employing relatively short infusions. Vogl et al.[17] compared a weekly 20-min hepatic artery administration with and without microspheres and reached a maximum-tolerated dose (MTD) of 1400 and 1800 mg/m^2, with myelosuppression as dose-limiting toxicity. In the study of Tse et al.[18] gemcitabine was administered at a fixed rate infusion (10 mg/min/m^2) with an MTD of 1500 mg/m^2 at an infusion time of 150 min which was associated with myelosuppression (Grade 2 thrombocytopaenia) and an increase in liver enzymes. Hepatic extraction at this schedule was highly variable, which was explained by saturation of dCDA; however, only pharmacokinetics of gemcitabine were performed but not of dFdU, which would have given information on this aspect.

Previously we have shown a strong schedule dependence of the anti-tumour activity of gemcitabine,[19,20] which can be explained by the rate-limiting activity of dCK.[12] Prolonged *in vitro* exposure and a long infusion of gemcitabine up to 24 h, with low peak plasma concentrations, could optimise the use of the full capacity of this enzyme. A preclinical study in mice bearing the colon 26-10 murine colon carcinoma showed a considerable increase in the gemcitabine efficacy by using a weekly 24-h schedule.[19,20] In addition, we have observed earlier that otherwise gemcitabine-insensitive tumours can be made sensitive by a prolonged exposure as can be achieved using a 24-h infusion.[19] Together with the preference of liver metastases over normal liver to activate gemcitabine,[14] a 24-h infusion can be beneficial to tumours normally not sensitive to gemcitabine. Several phase I and II clinical studies have applied 24-h intravenous infusion of gemcitabine weekly × 3, every 4 weeks. The MTD varied from 100 mg/m^2 to 180 mg/m^2 with myelosuppression being the major dose-limiting toxicity.[21–24]

We combined both features, HAI of gemcitabine and a 24-h schedule, and determined the toxicities and the MTD of gemcitabine when given by 24-h HAI, weekly × 3, every 4 weeks. Other objectives were to identify any potential anti-tumour activity of this regimen, as well as to compare the pharmacokinetics of gemcitabine when delivered by HAI and intravenous infusion. This was accompanied by measurements of dFdU to determine whether dCDA-mediated deamination was saturated. Based on earlier phase I and II studies of 24-h intravenous infusion the starting dose level was chosen as 75 mg/m^2/24 h.[21]

2. Patients and methods

2.1. *Patient selection*

Patients with a pathologically confirmed solid tumour metastatic to the liver that was not amenable to surgery were eligible for inclusion. The presence of minimal extrahepatic sites of disease was only allowed if the bulk of the disease was in the liver. Patients had to be between 18 and 70 years of age, have a World Health Organisation (WHO) performance status $\leqslant 2$ and have a life expectancy $\geqslant 3$ months. The anatomy of the liver arterial supply, evaluated by angiography, had to allow adequate liver perfusion by HAI through an implantable arterial access device. In addition, patients were required to have a white blood cell (WBC) count $\geqslant 3.0 \times 10^9$/L, a platelet count $\geqslant 100 \times 10^9$/L, serum bilirubin $\leqslant 25$ μmol/L, serum transaminases <2 times the upper limit of normal, a prothrombin time $\leqslant 1.5 \times$ the upper limit of normal, a serum creatinine $\leqslant 120$ μmol/L and/or a creatinine clearance $\geqslant 60$ ml/min. Previous radiotherapy or chemotherapy had to be discontinued at least 4 weeks before study entry. Patients were excluded if any of the following applied: prior therapy with gemcitabine, symptomatic central nervous system metastases, severe infection and uncontrolled non-malignant systemic disease. Women were ineligible if they were pregnant or were breast feeding. Written informed consent was required from all patients. The study was approved by the Medical Ethical Committee of the VU University Medical Center.

2.2. *Treatment plan*

Gemcitabine was given as a 24-h infusion weekly for 3 weeks followed by 1 week rest, which was one cycle of therapy. The

starting dose was 75 mg/m^2. During the first cycle, patients alternately received the first gemcitabine administration by intravenous infusion and the second gemcitabine administration by HAI or vice versa. This was done in order to perform pharmacokinetics. From the third administration of the first cycle on, gemcitabine was always given by HAI. The planned dose levels were 75 mg/m^2, 135 mg/m^2, 180 mg/m^2, 240 mg/m^2 and, if needed, additional dose escalation steps. Three consecutive patients had to be treated at each dose level. If no serious toxicity occurred, dose escalation was performed. Intrapatient dose escalation was not permitted. When one grade 4 toxicity or two grade 3 drug-related toxicities occurred at a dose level (excluding alopecia, nausea and vomiting), three additional patients were entered at that dose level. Dose-limiting toxicity (DLT) was defined as grade 4 haematologic toxicity or grade 3–4 non-haematologic toxicity (excluding alopecia, and nausea and vomiting) during any cycle of therapy. The MTD was defined as the highest dose level with DLT in $\geqslant$2 of 6 patients. If a patient developed grade 3–4 haematologic or non-haematologic toxicity, dose administration had to be postponed until recovery for a maximum of 2 weeks. This also applied for >grade 2 mucositis and for a transaminase increase >2.5 times the pretreatment values. The patient went off study, if the toxicity did not resolve within 2 weeks. The gemcitabine dose was reduced with 25% in case of continued treatment after recovery from grade 4 haematologic toxicity or from non-haematologic toxicities. A maximum of two dose reductions was allowed.

2.3. *Drug administration*

All patients underwent percutaneous implantation of an intra-arterial catheter connected to a subcutaneous infusion port (Port-A-Cath; SIMS Deltec Inc., St. Paul, MN). The tip of the catheter was placed in the hepatic artery, using the left subclavian artery as access. Before every cycle, the correct positioning of the catheter tip was ascertained by an abdominal X-ray. Gemcitabine (Gemzar; Eli Lilly & Co., Indianapolis, IN) was supplied in sterile vials containing 200 mg or 1000 mg. The appropriate amount of gemcitabine was dissolved in 1000 ml of 0.9% NaCl for administration by a bedside pump or in 250 ml of 0.9% NaCl for portable pumps and was infused over 24 h. On day 1 and day 8 of the first cycle, all patients were hospitalised for pharmacokinetics. Thereafter treatment was given on an ambulatory basis using portable pumps. Patients received no prophylactic anti-emetic therapy. If nausea or vomiting occurred, patients were given metoclopramide or ondansetron. Prophylactic administration of haematopoietic growth factors was allowed in case of severe neutropaenia, lasting for $\geqslant$1 week and/or accompanied by infection.

2.4. *Toxicity and response evaluation*

Pretreatment evaluation consisted of a medical history and physical examination, a complete blood cell count, total bilirubin, γ-glutamyltransferase (γ-GT), alkaline phosphatase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), prothrombin time, serum creatinine and creatinine clearance. These tests were repeated before each cycle. Body weight and performance status were assessed before each dose administration, and experienced side-effects, complete blood cell counts and liver enzymes were monitored weekly. Also, chest X-ray, abdominal CT-scan and ECG were performed before the first cycle and were repeated every two cycles, except for the ECG, which was only repeated if necessary. Tumour measurements were carried out every two cycles, and responses were evaluated according to the WHO criteria.[25] Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 1).[26]

2.5. *Pharmacokinetic analysis*

All patients underwent blood sampling on day 1 and day 8 of the first cycle to compare the pharmacokinetics of 24-h gemcitabine administration when given by HAI and by intravenous infusion. Blood samples (10 ml) were drawn just before gemcitabine infusion, at 2, 4 and 24 h during infusion, and at 0.5, 2 and 4 h postinfusion. This PK sampling schedule was rather restricted; however, if an earlier time point would also have been chosen during infusion, only a 5% difference in AUC would have been found. The blood specimens were collected in heparinised tubes containing 25 μl tetrahydrouridine (10 mg/ml) to prevent deamination of gemcitabine, and were placed on ice immediately after collection. Plasma was obtained by centrifugation of the samples at 4000 rpm for 5 min at 4 °C and was stored at –20 °C until analysis. For gemcitabine analysis, a sensitive liquid chromatographic/atmospheric pressure chemical ionisation tandem mass spectrometric method (LC–APCI-MS/MS) was used to enable the determination of very low gemcitabine concentrations.[27] Briefly, plasma was mixed with $^{15}N_3$-deoxycytidine (10 g/ml), which was used as the internal standard. Isopropyl alcohol was added to precipitate plasma proteins. After centrifugation, the supernatant was freeze-dried and reconstituted in ethyl acetate and Milli-Q water, permitting back-extraction into water. The aqueous layer was used for analysis with the LC–APCI-MS/MS system (a Perkin–Elmer series 200 HPLC coupled with a SCIEX API 3000 mass spectrometer). Chromatographic separation on a Sperisorb-phenyl-column was achieved with an isocratic formic-acid/acetonitrile mobile phase. Ions of gemcitabine (264/112 *m*/*z*) were detected and quantified with an accuracy, precision and limit-of quantification of: 99.8%, ±7.9% and 19 nM, respectively. The method was linear from 19 nM up to 2 μM. More details have been described elsewhere.[27] For dFdU analysis, a standard high performance liquid chromatography method was used as described previously.[28] Pharmacokinetic parameters, including the area under the plasma-concentration-versus-time curve from time 0 to the end of infusion (AUC_{0-24h}) and the maximum plasma concentration (C_{max}), were determined using the WinNonlin computer program (version 1.5, Scientific Consulting Inc., Apex, NC). The hepatic extraction of gemcitabine was calculated using the formula: $1 - (AUC_{0-24h}$ HAI/AUC_{0-24h} IV), in which AUC_{0-24h} HAI and AUC_{0-24h} IV represent the area under the plasma-concentration-versus-time curves from time 0 to 24 h for gemcitabine given by hepatic arterial infusion and intravenous infusion, respectively.[29] However, despite the use of this highly sensitive assay, postinfusion data points

were often not detectable after 2 h for the HAI. When calculating $AUC_{infinity}$ a minimum of three data points is necessary to achieve a reliable infinity end-point. This meant that the $AUC_{infinity}$ for the HAI samples could not be determined with any degree of reliability. Statistical analysis was performed with Microsoft Excel software (Microsoft Office XP 2003; Microsoft, Redmond, WA). Pearson's correlation coefficient (r) was used for the relationship between the dose and the pharmacokinetic parameters: C_{max} and AUC_{0-24h}. To compare the pharmacokinetic parameters of HAI with those of intravenous infusion, a Student's t-test for paired samples was used. The level of significance was set at 0.05. All tests for significance were two tailed. In order to evaluate the relation between dose and liver extraction we performed an ANOVA analysis, both one way and using a multiple comparison according to the Bonferroni principle. In order to evaluate a relationship between toxicity and pharmacokinetics we calculated the percentage decrease in thrombocytes: $1 - [(\text{thrombocytes}_{\text{day 0}} - \text{thrombocytes}_{\text{nadir}})/\text{thrombocytes}_{\text{day 0}}] \times 100\%$.

The nadir of the first cycle and the nadir over the whole treatment were evaluated in relation to PK parameters: AUC_{0-24h} HAI, AUC_{0-24h} IV, C_{max} HAI and C_{max} IV for both gemcitabine and its metabolite dFdU using the Spearman nonparametric ranking test.

3. Results

3.1. Patients

Thirteen patients were entered in this study. Patient characteristics are described in Table 1. All patients had solid tumours of the gastrointestinal tract. Three patients had minimal extra-hepatic disease. The total number of cycles given was 55. The median number of cycles per patient was 2 (range 0–11) (Table 2). Treatment was given at three consecutive dose levels: four patients at the 75 mg/m^2 dose level, three patients at the 135 mg/m^2 dose level and six patients at the 180 mg/m^2 dose level. One patient with colorectal cancer had an obstruction of the hepatic artery before gemcitabine could be given by HAI. This patient only received one administration of gemcitabine intravenously (at the 75 mg/m^2 dose level) and was therefore not included in the analysis of the study results. An additional patient was therefore entered in that cohort.

Table 1 – Patient characteristics.

Variable	No. of patients
Total no. of patients (evaluable)	13 (12)
Male/female	9/4
Age, years	
Median	60
Range	40–70
Performance status (WHO) 0/1/2	5/8/0
No. of prior chemotherapy regimens	
0	4
1	6
2	1
3	2
Primary tumour	
Colorectal	7
Liver	3
Pancreas	2
Biliary tract	1

3.2. Toxicity

Twelve patients were evaluable for toxicity as listed in Table 3. The principal haematologic toxicity was thrombocytopaenia, observed in eight of the 12 patients. The nadir platelet count occurred at a median of 14 d (range: 7–17 d) in each cycle and was reversible in all patients, usually within 1 week. Toxicity increased with dose. Grade 3 thrombocytopaenia developed in one of the three patients at the 135 mg/m^2 dose level and in one of the six patients at the 180 mg/m^2 dose level, both at day 15 of cycle 1 in which the HAI was given at day 1 and the intravenous infusion at day 8. Grade 3 thrombocytopaenia reoccurred in the patient at the 135 mg/m^2 dose level at day 15 of cycle 9. Grade 4 thrombocytopaenia was seen in two patients at the 180 mg/m^2 dose level. In one patient thrombocytopaenia was observed at day 15 of cycle 1, after giving the HAI at day 1 and the intravenous infusion at day 8. In the other patient grade 4 thrombocytopaenia developed at day 8 of cycle 3. Therefore, the MTD was determined to be 180 mg/m^2 with thrombocytopaenia as the dose-limiting toxicity. Grade 4 thrombocytopaenia was complicated by intermittent epistaxis in one patient and for that reason a platelet transfusion was given. After an initial recovery within 1 week, grade 3 thrombocytopaenia recurred the week thereafter and therefore this patient went off study. The second patient with grade 4 thrombocytopaenia did not experience complications and, after a week postponement, was able to continue treatment with a 25% dose-reduction. Other severe myelosuppression was rare: one patient at the 135 mg/m^2 dose level with grade 3 thrombocytopaenia also developed grade 3 neutropaenia, and one patient at the 180 mg/m^2 dose level with grade 3 thrombocytopaenia experienced grade 3 leucopaenia. No patients needed haematopoietic growth factors for neutropaenia or other types of haematologic toxicity.

Non-haematologic toxicity was generally mild to moderate. Grade 1–2 nausea and vomiting were experienced by eight and five patients, respectively. Transient elevations of the liver transaminases (ALT and/or AST) were documented in eight patients at the two highest dose levels, and included one patient with a grade 3 ALT elevation. Five of these patients had pre-existing elevated values. The development of grade 1–2 fever in absence of infection occurred in six patients. Mild (grade 1) flu-like symptoms were experienced by four patients. Other non-haematologic toxicities included grade 1 and grade 3 diarrhoea, developed by one patient at the 75 mg/m^2 and 180 mg/m^2 dose level, respectively. Grade 1–2 stomatitis was observed in one patient at the 135 mg/m^2 dose level and in one patient at the 180 mg/m^2 dose level.

3.3. Pharmacokinetics and pharmacodynamics

Pharmacokinetic analysis was performed in 12 patients. The pharmacokinetic data are summarised in Table 4. For HAI, the C_{max} and the AUC_{0-24h} of both gemcitabine and dFdU

Table 2 – Extent of exposure to study therapy.

Dose (mg/m^2)	Patients	Number of cycles		Delay of therapy due to grade 3–4 thrombocytopaenia		Patients with dose reduction (reduced cycles per patient)
		Median per patient (range)	Total	Number of patients	Number of weeks per patient	
75	4	2 (0–4)	8	0	0	0
135	3	8 (2–10)	20	1	1	0
180	6	2 (2–11)	27	3	1/1/2	2 (1/1)

Table 3 – Incidence and grade of principal haematologic and non-haematologic toxicities (all cycles).

Toxicity	75 mg/m^2 dose ($n = 3$)				135 mg/m^2 dose ($n = 3$)				180 mg/m^2 dose ($n = 6$)			
	Grade				Grade				Grade			
	1	2	3	4	1	2	3	4	1	2	3	4
Haematologic toxicity												
Thrombocytopaenia	0	0	0	0	2	0	1	0	1	1	1	2
Leucopaenia	0	0	0	0	1	1	0	0	2	1	1	0
Neutropaenia	0	0	0	0	0	0	1	0	1	3	0	0
Anaemia	1	0	0	0	1	0	0	0	2	1	0	0
Non-haematologic toxicity												
Nausea	2	0	0	0	1	1	0	0	2	2	0	0
Vomiting	2	0	0	0	0	0	0	0	2	1	0	0
ALT elevation	0	0	0	0	1	2	0	0	1	3	1	0
AST elevation	0	0	0	0	0	1	0	0	1	4	0	0
Fever without infection	0	0	0	0	2	1	0	0	1	2	0	0
Flu-like symptoms	1	0	0	0	2	0	0	0	1	0	0	0
Fatigue	0	1	0	0	0	1	0	0	0	0	1	0

Table 4 – Pharmacokinetic parameters of gemcitabine and dFdU for HAI and intravenous infusion.

Dose (mg/m^2)	No. of patients	Gemcitabine		dFdU	
		C_{max} (nM) Mean ± SD Median (range)	$AUC_{0–24h}$ (nmol × h/L) Mean ± SD Median (range)	C_{max} (μM) Mean ± SD Median (range)	$AUC_{0–24h}$ (μmol × h/L) Mean ± SD Median (range)
HAI					
75	3	25.8 ± 12.7 18.4 (14.1–44.8)	385.6 ± 165.1 414.9 (138.0–604.0)	3.7 ± 0.5 3.1 (3.1–4.2)	36.2 ± 10.8 30.6 (25.7–52.4)
135	3	79.9 ± 38.4 63.6 (38.7–137.5)	1247.4 ± 613.4 1049.0 (525.7–2167.5)	11.4 ± 3.4 10.0 (7.6–16.5)	112.5 ± 30.4 106.7 (72.7–158.1)
180	6	135.8 ± 35.2 144.4 (78.2–176.7)	2032.7 ± 634.3 1771.0 (1140.7–3003.7)	11.0 ± 2.9 11.2 (7.1–14.7)	118.0 ± 32.1 117.8 (73.5–172.7)
Intravenous infusion					
75	3	229.0 ± 37.4 209.6 192.3–285.2)	3526.4 ± 400.7 3580.5 (2925.4–4073.3)	4.3 ± 0.4 4.5 (3.7–4.6)	41.6 ± 3.9 44.0 (35.8–45.1)
135	3	263.6 ± 43.6 267.6 (198.2–325.0)	4818.5 ± 1523.6 4030.8 (3320.7–7103.9)	9.9 ± 2.8 8.2 (7.4–14.1)	107.2 ± 33.5 84.7 (79.4–157.4)
180	6	292.5 ± 59.1 311.4 (148.5–403.8)	4863.4 ± 2165.8 4779.5 (1979.2–7517.5)	12.5 ± 3.7 11.7 (8.0–18.6)	131.4 ± 35.7 124.5 (93.4–178.3)

increased linearly with the dose (gemcitabine: $r = 0.764$, $P = 0.004$ and $r = 0.742$, $P = 0.006$, respectively, and dFdU: $r = 0.667$, $P = 0.018$ and $r = 0.687$, $P = 0.014$, respectively). For intravenous infusion, a similar dose-dependent increase was observed for the C_{max} and the $AUC_{0–24h}$ of dFdU ($r = 0.720$, $P = 0.008$ and $r = 0.748$, $P = 0.005$, respectively), but not for the C_{max} and the $AUC_{0–24h}$ of gemcitabine ($r = 0.379$, $P = 0.225$ and $r = 0.408$, $P = 0.188$, respectively). Figs. 1 and 2 show the mean plasma-concentration-versus-time curves of gemcitabine and dFdU. Intrapatient comparison of HAI and intravenous infusion demonstrated a significantly lower C_{max} ($P = 0.000$) and $AUC_{0–24h}$ ($P = 0.000$) of gemcitabine for HAI. Thus, gemcitabine was extracted relatively well with mean hepatic extraction ratios at the 75, 135 and 180 mg/m^2 dose

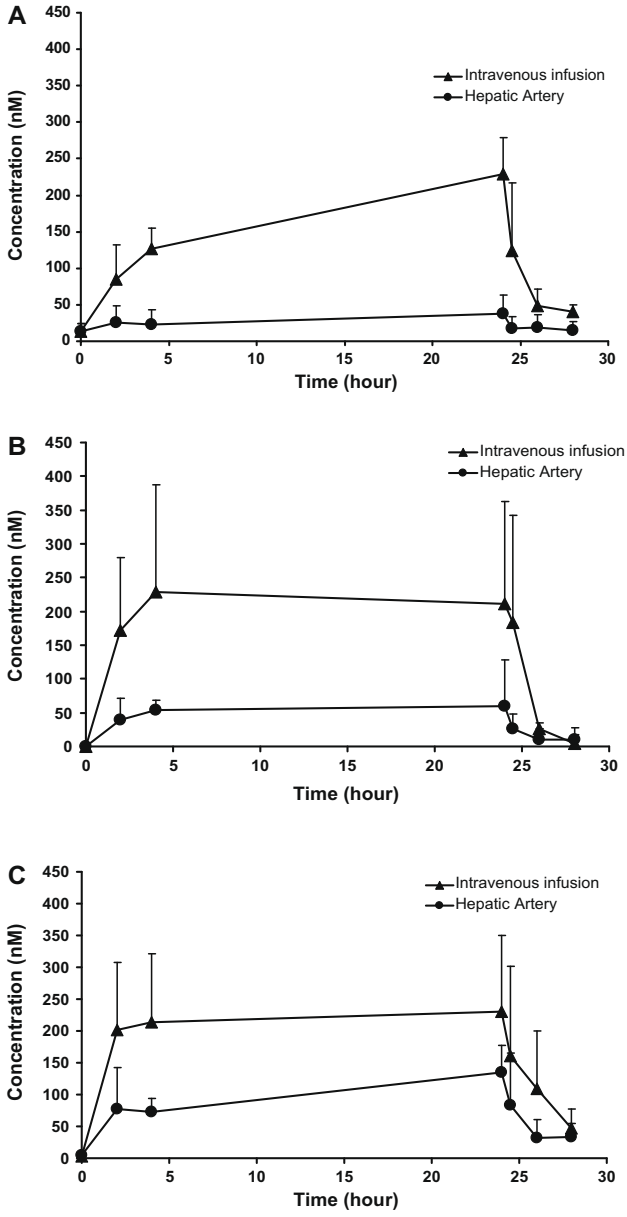

Fig. 1 – The mean (±SD) plasma-concentration-versus-time curves of gemcitabine for HAI and intravenous infusion at the 75, 135 and the 180 mg/m^2 dose level (A, B and C, respectively).

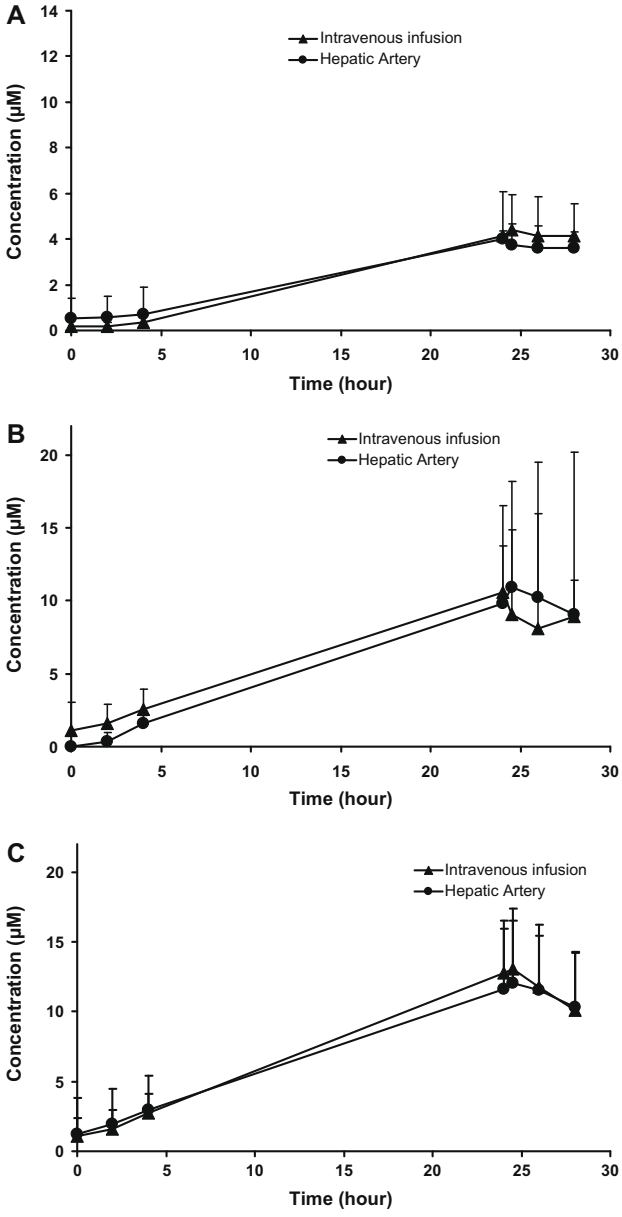

Fig. 2 – The mean (±SD) plasma-concentration-versus-time curves of dFdU for HAI and intravenous infusion at the 75, 135 and the 180 mg/m^2 dose level (A, B and C, respectively).

level of: 0.89 (range 0.83–0.97), 0.75 (range 0.68–0.87) and 0.55 (range 0.22–0.83), respectively. The hepatic extraction ratios decreased linearly with increasing dose ($r = -0.664$, $P = 0.019$), (Fig. 3). The ANOVA analysis demonstrated a significant difference between the AUC for the HAI doses of 75 and 180 mg/m^2 groups, but not between the doses of 75 and 135 mg/m^2, and 135 and 180 mg/m^2. Also, no significant difference was found between the intravenous doses. For the liver extraction, ANOVA analysis showed a tendency towards significance ($P = 0.066$). In contrast, for the C_{max} and the AUC_{0-24h} of dFdU, there was no significant difference between HAI and intravenous infusion ($P = 0.476$ and $P = 0.499$, respectively). The latter is likely be due to the high activity of cytidine deaminase in the liver, the major organ responsible for

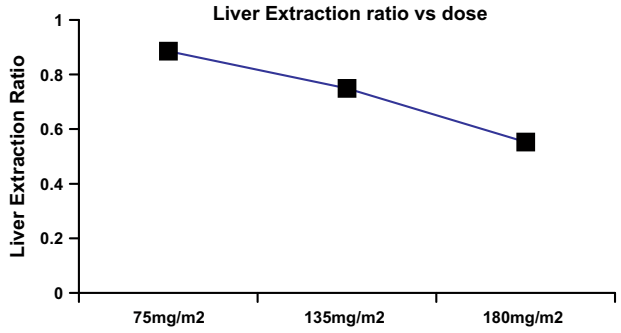

Fig. 3 – Relation between the hepatic extraction coefficient and dose.

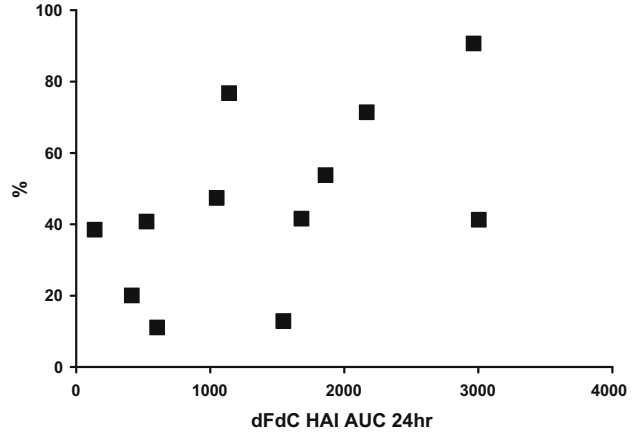

Fig. 4 – Correlation between the $AUC_{0–24h}$ HAI gemcitabine and the dose-limiting toxicity, thrombocytopaenia, given as percentage decrease in thrombocytes. Correlation coefficient (Spearman) was 0.0239 (one-tailed test). No correlation was found with the $AUC_{0–24h}$ IV gemcitabine.

the elimination of gemcitabine. Due to a first-pass effect, deamination of intravenously administered gemcitabine occurs as rapidly as compared with gemcitabine given by HAI.

In order to determine whether any pharmacokinetics-pharmacodynamics relationship was present, and whether this was influenced by the time-course of the toxicity, we evaluated the nadir of thrombocytopaenia in cycle 1 and over all cycles in relation to the HAI and IV pharmacokinetic parameters (Fig. 4). The $AUC_{0–24h}$ and the C_{max} for gemcitabine were significantly correlated with thrombocytopaenia observed in Cycle 1 ($P = 0.0239$ and $P = 0045$, respectively). A similar relationship was observed for the nadir for thrombocytopaenia over cycles 1–6 ($P = 0.0256$ and $P = 0.0688$, respectively). Interestingly a tendency towards significance was found for the $AUC_{0–24h}$ and C_{max} for dFdU and toxicity in cycle 1 ($P = 0.0591$ and $P = 0.0681$, respectively). None of the pharmacokinetic parameters (AUC and C_{max} for gemcitabine and dFdU) for the IV administration was related to toxicity.

3.4. *Tumour response*

Twelve patients were evaluable for tumour response. No complete or partial responses were observed. However, over the entire dose range (75–180 mg/m^2), seven patients had stable disease for a median duration of 9 months (range: 2–11 months). They had the following tumour types: colorectal cancer (three patients), hepatocellular carcinoma (three patients) and cholangiocarcinoma (one patient). The patients with hepatocellular carcinoma and cholangiocarcinoma experienced a remarkable longer period of stable disease (range: 9–11 months) than the patients with colorectal cancer (range: 2–4 months).

4. Discussion

In this phase I and pharmacokinetic study, the MTD of gemcitabine given by weekly 24-h HAI was 180 mg/m^2 with thrombocytopaenia as the dose-limiting toxicity. Other toxicities were mild to moderate. In two previous studies on HAI of gemcitabine myelosuppression was dose-limiting as well, including thrombocytopaenia.[17,18] In both studies a clinical benefit was observed in patients with cholangiocarcinoma. However, in one study the administration period was only 20 min.[17] In the other study the infusion period at an infusion rate of 10 mg/min/m^2 was 150 min and at 2.5 mg/min/m^2 it was 400 min,[18] which yielded MTDs very close to that observed for gemcitabine administered intravenously (1500 and 1000 mg/m^2, respectively). Weekly 24-h infusions were studied in a phase I study in which gemcitabine was given by weekly 24-h intravenous infusion in patients with inoperable NSCLC. The MTD was 180 mg/m^2 with neutropaenia and lethargy as the principal toxicities.[21] In the current study, neutropaenia was mild to moderate, no lethargy was experienced and HAI of gemcitabine did not allow a higher MTD due to dose-limiting thrombocytopaenia. The inability to increase the MTD of gemcitabine by HAI may be caused by the fact that in the current study most patients had received prior chemotherapy, while in the published study on 24-h intravenous infusion all patients were chemotherapy naïve. The administration of gemcitabine via the intravenous route on day 8 of cycle 1 might have caused the early occurrence of grade 3–4 thrombocytopaenia in 3 patients, because of a higher systemic exposure. At 180 mg/m^2 this happened in two patients and only in cycle 3 in the other patient. This might have underestimated the DLT of HAI gemcitabine, but we believe that this did not compromise the estimation of the MTD. Further evidence for a minor role of the 24-h IV infusion regarding toxicity was obtained by the lack of correlation (all P-values > 0.215) between the IV pharmacokinetic parameters for gemcitabine and toxicity, even when only toxicity in cycle 1 was considered. The pharmacokinetic/pharmacodynamic correlation was found for the HAI parameters, underlining that the IV infusion did not play an important role in toxicity.

More recently, a pilot study was published on weekly 24-h intravenous infusion of gemcitabine in pre-treated and chemotherapy-naïve patients with biliary tract and pancreatic cancer. This study reported an MTD of 100 mg/m^2, especially in pre-treated patients. The dose-limiting toxicity was neutropaenia in chemotherapy-naïve patients and thrombocytopaenia in pre-treated patients. In the pre-treated patients, grades 3 and 4 thrombocytopaenia were documented in three of the eight evaluable courses at the 150 mg/m^2 dose level, respectively.[22] In comparison with the latter study, the increased MTD of gemcitabine, when given by 24-h HAI, suggests that systemic toxicity may be reduced by HAI.

In none of the above-mentioned studies,[21–24] the pharmacokinetics of 24 h gemcitabine infusion was reported, while in one of the studies on HAI gemcitabine plasma levels of gemcitabine were reported but not those of dFdU.[18] In the latter study a highly variable extraction of gemcitabine was found with ratios of gemcitabine levels of HAI to IV ranging from 0.09 to 11 (median 0.95). In order to increase the hepatic extraction the length of duration was prolonged to 400 min by decreasing the rate to 2.5 mg/min/m^2. Plasma concentrations for gemcitabine varied from 0.09 to 4.66 μg/ml (0.3–15.5 μM) for IV and from 0.34 to 6.53 μg/ml (0.9–21.8 μM) for HAI after 1 h. It was hypothesised that a longer infusion

would increase the hepatic extraction but in their cohort no evidence was found. However, in our study the pharmacokinetics of gemcitabine showed a significantly lower C_{max} and AUC_{0-24h} for HAI than intravenous infusion, which was found in each patient. The corresponding mean hepatic extraction ratios decreased with increasing dose from 0.89 to 0.55. This explains the relatively low extraction at the high doses used in the study of Tse et al.[18] This decrease in hepatic extraction might be caused by saturation of dCDA in the liver or by saturation of the gemcitabine transport across the cell membrane of liver cells.[30] Nevertheless, gemcitabine was extracted relatively well by the liver, with hepatic extraction ratios comparable to those described for FUDR (0.69–0.92) and fotemustine (0.36–0.86), and higher than the hepatic extraction ratios of doxorubicin (0.45–0.50), 5-FU (0.22–0.45), and cisplatin (0.00–0.50).[31–34] This implicates a strong pharmacokinetic rationale for the administration of gemcitabine by HAI. Objective tumour responses were not observed in this study. However, seven of the 12 evaluable patients had stable disease, including four patients with disease stabilisation for >9 months: three of them with hepatocellular carcinoma and one patient with cholangiocarcinoma. Further investigation of the efficacy of gemcitabine administered by 24-h HAI is warranted in these two tumour types, also considering the clinical benefit against these tumours found in the two other studies with HAI of gemcitabine.[17,18]

In conclusion, the MTD of gemcitabine given by 24-h HAI was 180 mg/m^2 with thrombocytopaenia as the dose-limiting toxicity. The extraction of gemcitabine by the liver was relatively high, with significantly lower systemic gemcitabine plasma concentrations for HAI than intravenous infusion. There was evidence for potential therapeutic efficacy, in terms of prolonged disease stabilisation, although it should be realised that these patients were pre-treated. These clinical and pharmacological results warrant further investigation of the administration of gemcitabine by 24-h HAI, possibly in combination with systemically administered drugs targeted against tyrosine kinases. The recommended dose for consecutive phase II studies is 135 mg/m^2.

Conflict of interest statement

None declared.

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