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Clinical phase II study and pharmacological evaluation of rubitecan in non-pretreated patients with metastatic colorectal cancer—significant effect of food intake on the bioavailability of the oral camptothecin analogue

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Abstract

A randomised, open label phase II study was performed in patients with advanced colorectal cancer to evaluate the safety, toxicity and antineoplastic activity of the topoisomerase I-inhibitor rubitecan. A cross-over design was chosen to determine the intra-patient variation of the bioavailability and pharmacokinetics of the anticancer agent depending on the timing of food intake in relation to the oral drug administration. Patients with previously untreated metastatic disease received two single oral doses of rubitecan 1.5 mg/m^2 for assessment of the pharmacokinetics. They were randomised to have the first administration either after an overnight fasting period or immediately after a high calorie breakfast, and crossed over to the alternative schedule after a one-week washout period. After completion of the pharmacokinetic sampling, treatment continued with rubitecan given orally at a dose of 1.5 mg/m^2/day, to be increased up to 2.0 mg/m^2/day, under fasting conditions for 5 consecutive days per week until disease progression. 19 patients entered the trial after informed consent was obtained. A total number of 35 treatment cycles (median 2, range 1–4) were administered. All patients were evaluable for safety. The toxicity profile of rubitecan was generally mild to moderate, with sporadic cases of grade 4 toxicities (Common Toxicity Criteria (CTC) version 2.0) diarrhoea, leucopenia and neutropenia. None of 15 evaluable patients achieved an objective response. The majority had early disease progression. 14 patients were evaluable for pharmacokinetic analysis. The bioavailability of rubitecan was found to be strongly dependent on the timing of food intake with a fasted-to-fed ratio for C_{max} of 1.98 (two-tailed $P<0.001$; ANOVA), T_{max} 0.49 ($P<0.001$), $AUC_{0-8\ h}$ 2.52 ($P<0.001$) and $AUC_{0-24\ h}$ 1.64 ($P=0.003$). Rubitecan is well tolerated, but clinically inactive in colorectal cancer at the currently recommended dose and schedule. The bioavailability is strongly dependent on the timing of food intake in relation to the oral administration of the drug. The topoisomerase I-inhibitor should be administered under fasting conditions to achieve adequate drug exposure in future prospective trials in other tumour types.

Keywords: Bioavailability; Colorectal cancer; Food effects; Oral chemotherapy; Rubitecan; Topoisomerase I inhibitors

1. Introduction

The parent compound of all topoisomerase I-inhibiting anticancer agents is 20(S)-camptothecin, a plant alkaloid initially isolated from the Chinese tree *Camptotheca acuminata* (*Nyssaceae*) and structurally identified by Wall and colleagues [1]. This compound was abandoned during early stages of its clinical testing due to significant toxicity, until interest was revived by the discovery of its unique mode of action, the interaction with DNA-topoisomerase I [2]. More recently, two water-soluble derivatives of 20(S)-camptothecin, irinotecan and topotecan, were found to be safe and active in

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a number of human malignancies, and are now commonly used for intravenous palliative treatment of colon, rectal, lung and ovarian cancer. New insoluble topoisomerase I-inhibiting agents are in advanced stages of clinical development, including rubitecan (9-nitro-20(S)-camptothecin, RFS-2000; see Fig. 1) and exatecan (9-amino-camptothecin, DX-8195f). These compounds have considerable cytotoxic activity in a wide array of preclinical models including various human tumour xenografts [3,4]. It was found that a small amount of rubitecan actually converts to 9-amino-camptothecin in human tissue and appears to be more stable, while being equivalent in preclinical models in tumour inhibitory efficacy [5,6]. Furthermore, rubitecan has the potential advantage of oral bioavailability in various species. In mice, the achieved plasma concentrations with oral treatment are above those required to inhibit tumour growth *in vitro*. According to the results of recent phase I/II studies, oral rubitecan should be administered once daily at a dose of 1.0–2.0 mg/m^2/day for 4–5 consecutive days on a weekly basis [7]. Clinical responses have been observed in patients with breast, ovarian and pancreatic cancers and in haematological malignancies [7,8]. The current development of rubitecan is focused on phase III studies in pancreatic cancer, stimulated by a number of tumour regressions, as well as clinical benefit and quality-of-life gain in patients with this highly chemoresistant tumour type, and a broad phase II evaluation in various malignancies in both Europe and North America [8].

The present pharmacological and clinical phase II study was performed to evaluate the safety and antitumour activity of rubitecan in chemotherapy-naïve patients with metastatic colorectal cancer and to characterise the oral bioavailability of rubitecan capsules in relation to the timing of food intake by intrapatient comparison. Colorectal cancer is known to be highly susceptible to the inhibition of topoisomerase I, and a number of colorectal cancer cell lines and xenografts were sensitive to the treatment with rubitecan in preclinical testing.

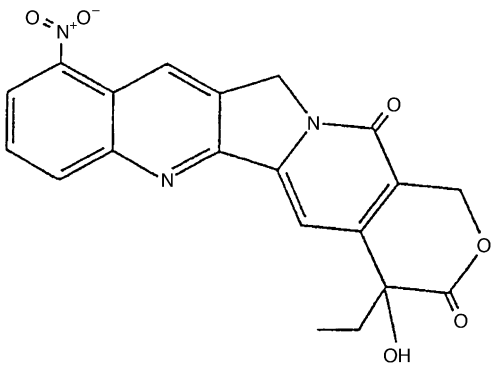

Fig. 1. Rubitecan.

2. Patients and methods

2.1. Study objectives

The study was initiated to determine if partial or complete responses can be achieved in patients with advanced colorectal cancer, to assess the probability of an actual response rate warranting further evaluation of the therapeutic effectiveness in cases that responses are observed, and to further characterise the toxicity pattern of rubitecan. The trial was performed in Hannover, Germany and Edegem, Belgium. A further endpoint was to determine the oral bioavailability if rubitecan was administered immediately after a high-calorie breakfast, compared with the administration after an overnight fasting period. The main pharmacokinetic endpoints were the area under the curve (AUC) exposure and the maximum plasma concentration (C_{max}) of rubitecan after two single exposures with a 1 week interval.

2.2. Patient selection criteria

Patients had to have histologically-verified, locally advanced, unresectable or metastatic colorectal cancer with at least one bidimensionally measurable target lesion. The minimum size of measurable lesions was defined as 2.5 cm in one diameter, with the exception of lung lesions where a size of 1.5 cm was required. Histological proof of malignancy was obtained in cases of a single malignant deposit. Patients were required to be at least 18 years old, have a good performance status World Health Organization (WHO) 0–2), a life expectancy of at least 3 months, and adequate bone marrow, renal and liver function (neutrophils $\geqslant 1.5\times10^9$ cells/l, platelets $\geqslant 100\times10^9$ cells/l, creatinine $\leqslant 177$ μmol/l, bilirubin $\leqslant 1.5\times$the upper limit of normal (UNL), asparate aminotransferase (ASAT) and alanine aminotransferase (ALAT) $\leqslant 3\times$UNL in the absence or $\leqslant 5\times$UNL in the presence of liver metastases).

Prior chemotherapy for advanced disease was not allowed. A minimum of 1 month had to have elapsed between the end of prior adjuvant radiotherapy, 3 months in cases of adjuvant pretreatment with the monoclonal antibody edrecolomab (17-1A), and 1 year in cases of previous adjuvant chemotherapy based on 5-fluorouracil, folinic acid or levamisole. Pre-treatment with topoisomerase I-inhibitors was not allowed. Patients had to be able to drink 3 l of fluids per day during treatment.

2.3. Ethical and methodological considerations

The study was carried out in accordance with the requirements of the Declaration of Helsinki. Written informed consent was obtained from all patients

according to German and Belgian law. Two independent ethics committees approved the study. Patient insurance was provided. The trial was co-ordinated and monitored by NDDO Oncology, Amsterdam, The Netherlands, and performed according to Good Clinical Practice standards. Standard Operating Procedures of NDDO Oncology were applied. Quality assurance was based on site source data verification and double entry into the data base of the biometrics department of NDDO Oncology.

2.4. Treatment

Rubitecan was formulated in hard gelatine capsules containing 0.5 or 1.25 mg of active drug and approximately 382 mg of lactose. The capsules were stored at 4–8 °C in an airtight, opaque container until use. After informed consent was obtained, all patients were randomised by a telephone procedure at NDDO Oncology to receive the first single dose of rubitecan on day −7 of the actual treatment period either with ('fed') or without ('fasted') breakfast. If randomised to start under fasted conditions, patients had their last meal at least 10 h prior to the first administration of the study drug, and were allowed to have their regular, non-defined breakfast within 3 h after taking the first dose of rubitecan 1.5 mg/m^2. After a 1-week washout period, the patients received the same dose after crossing over to the alternative breakfast scheme. The study-specific breakfast in the fed group consisted of 58% fat, 15% protein and 27% carbohydrate (1122 kcal) and was consumed under observation within 30 min immediately prior to the next single oral dose of rubitecan.

After completion of 24-h pharmacokinetic plasma sampling, patients continued to take rubitecan after an overnight fast (water allowed) at least 1 h prior to their regular breakfast (see Fig. 2). The starting dose of rubitecan was 1.5 mg/m^2 once daily for 5 consecutive days given on a weekly basis. The study protocol provided a dose-reduction scheme in cases of severe toxicity, and dose-escalation instructions based on the weekly assessment of the neutrophil and platelet counts. The dose was to be escalated up to 2.0 mg/m^2/day in cases of only minor haematological toxicity. Patients were instructed to drink 3 l of fluids per day to prevent chemical cystitis, which was reported in previous clinical trials with the agent. The treatment compliance was documented on patient diary cards. A period of 4 weeks was considered as one cycle of treatment. Patients with progressive disease were removed from the study. Patients achieving an objective response were allowed to remain on the study until disease progression or unacceptable toxicity occurred. In cases of disease stabilisation, the treatment could be discontinued after two treatment cycles. Ancillary treatments were given as medically indicated, but patients did not receive concurrent anticancer treatment, investigational agents or haematopoietic growth factors.

2.5. Study design and study parameters

The two-centre, open label randomised phase II study had a two-stage statistical design [9]. In the first stage, 14 evaluable patients were to be entered. If there were no response in this group, the trial would be terminated. This ensures that if the drug is active in at least 20% of patients, the chance of erroneously rejecting it after the first cohort is $<5\%$. In case objective responses were observed, the study was to continue up to a maximum number of 11 additional patients, depending on the number of responses observed. This allows the determination of the response rate with a standard error of <0.10.

The response assessment was performed at least every 8 weeks with clinical examination, computer tomography and/or magnetic resonance imaging based on WHO criteria. The serum tumour markers carcinoembryonic antigen (CEA) and CA 19.9 were assessed every 4 weeks. Early progression was defined as progression during the first cycle of treatment. The assessment of haematological toxicity was based on a weekly differential blood count. Serum chemistry, urine analysis, history and physical examination were performed every 4 weeks. Toxicity was assessed and graded according to National Cancer Institute (NCI) Common Toxicity Criteria (CTC) (version 2.0). The safety analysis included all of the patients enrolled in the study.

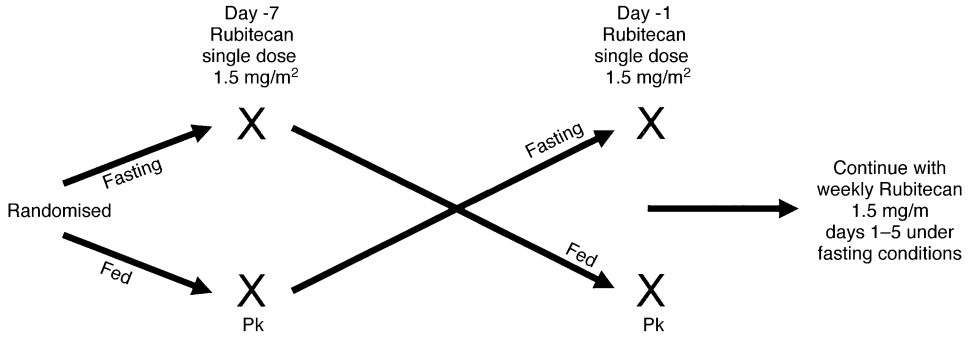

Fig. 2. Crossover design for intrapatient assessment of food effects on bioavailability. PK = Pharmacokinetic sampling over 24 h.

2.6. Pharmacokinetic blood sampling and high-performance liquid chromatography (HPLC) analysis

To determine the bioavailability and pharmacokinetics of rubitecan, the food effect was examined by a single-dose, two-period, two-treatment cross-over design. The minimum number of patients required for this purpose was estimated to be 14, based on an expected coefficient of variation due to an intrapatient variability of 15–20%. Patients for whom all required samples were obtained were considered evaluable for pharmacokinetics. Pharmacokinetic blood sampling was done immediately prior to dosing and 30 min, 1, 2, 3, 4, 6, 8 and 24 h after the administration of rubitecan on days −7 and +1 of treatment. Samples were immediately centrifuged and frozen at −70 °C. Plasma samples from both study sites were shipped on dry ice to the Netherlands Cancer Institute and stored at −70 °C until analysis.

Total concentrations (lactone and carboxylate forms) of rubitecan and the active metabolite 9-amino-camptothecin (data not shown) were determined using analytical methods developed by Rizzo (Institute for Drug Development, San Antonio, CA, USA) and validated by the Department of Pharmacy and Pharmacology at the Netherlands Cancer Institute [10–12]. A HPLC method with ultraviolet and fluorescence detection was used for the analysis. Reference standards for rubitecan and 9-amino-camptothecin were supplied by SuperGen, Pleasanton (CA, USA). Calibration standards were stored at 4–8 °C.

We did not attempt to study steady-state pharmacokinetics in this trial, which would have required repeated dosing during the sampling period and would have allowed a pharmacodynamic analysis.

2.7. Statistical analysis of pharmacokinetic data

The impact of food intake was assessed using four pharmacokinetic parameters, namely C_{max}, time to maximum drug concentration (T_{max}), $AUC_{0–8\ h}$ and $AUC_{0–24\ h}$. These parameters were obtained using a model-independent approach. C_{max} and T_{max} were generated directly from the experimental data. For rubitecan, the $AUC_{0–8\ h}$ and $AUC_{0–24\ h}$ were calculated by the trapezoidal rule. If the concentration at the last time point (24 h) was less than the lower limit of quantitation, extrapolation to 24 h was performed using the terminal rate constant.

The Statistical Package for the Social Sciences (SPSS) version 6.1.4 for Windows (Chicago, IL, USA) was used for the statistical analysis of the data-set. Each treatment course represented a two-period crossover design. The statistical test employed was an analysis of variance (ANOVA) model with period and regime as the main effects and the following interactions: regimen×period (carry over) and subject×sequence (interpatient variability). The parameters were logarithmically transformed to ensure the additivity of the model effects and to bring the distribution of the data close to a normal distribution. The null hypothesis of no difference between the two regimens was tested at a 5% level of significance. Classical 90% Confidence Intervals (CIs) were calculated for the tested parameters. According to defined criteria for assessing bio-equivalence, the mean log transformed parameters under the test conditions (i.e. fasted) should be within 80—125% of the parameters under reference conditions (i.e. fed) [13].

3. Results

3.1. Eligibility and feasibility of the treatment

Between 11/1999 and 07/2000, 19 patients were enrolled into the trial. All of the patients were fully eligible. 15 patients were evaluable for response, reasons for non-evaluability and further patient characteristics are given in Table 1. The cohort represented a typical, non-selected group of patients with metastatic colorectal cancer in the two participating institutions. A total number of 35 treatment cycles were administered, the median number of courses was 2 with a range of 1–4 per patient. A dose escalation of rubitecan based on the weekly differential blood count was administered in 10 patients. The drug treatment was temporarily omitted in only two cycles due to haematological toxicity. The patient diary cards indicated an excellent compliance with the treatment scheme. Gastrointestinal toxicity did not compromise the intake of the oral drug according to

Table 1
Patient characteristics

Gender (no. of patients)	
Male	10
Female	9
Age (years)	
Median (range)	59 (40–78)
Performance status	
WHO 0	12
WHO 1	7
Pre-treatment (no. of patients)	
Surgery	19
Adjuvant chemotherapy	4
Adjuvant radiotherapy	0
Palliative chemotherapy	0
Eligibility and evaluability (no. of patients)	
Eligible	19
Evaluable for response	15
Evaluable for toxicity	19
Evaluable for pharmacokinetics	14

WHO, World Health Organization.

the diary card assessment. Complete pharmacokinetics were performed in 14 patients, all of them were fully compliant with the given breakfast instructions as required by the protocol.

3.2. Toxicity

In general, the treatment was well tolerated, and the toxicity of rubitecan was fully reversible. The main haematological and non-haematological toxicities are summarised in Table 2. No treatment-related serious adverse event or treatment-related death occurred. Only one treatment cycle was discontinued early due to drug toxicity. All adverse events were mild to moderate, with the exception of one case of grade 4 diarrhoea, which was considered to be severe and drug related.

3.3. Response

Among the 15 evaluable patients, no objective responses were achieved (Table 3). The best response observed was short-lasting stable disease in 3 patients, who were taken off study due to disease progression after only two further cycles of treatment. None of our patients had a significant decrease in serum CEA or CA 19.9. Based on these results, the study did not re-open for further patient recruitment after the first step of accrual was achieved. All but 3, patients received immediate salvage chemotherapy, based on 5-fluorouracil/folinic acid, oxaliplatin or irinotecan.

Table 2
Toxicity according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) 2.0 (maximum toxicity per cycle, $n = 35$ cycles)

Adverse event	CTC Toxicity Grade				Grades 1–4
	1	2	3	4	
	Number of cycles				
Haemoglobin	6	8	1	0	15 (43%)
Nausea	11	3	0	0	14 (40%)
Lymphocytes	6	2	5	0	13 (37%)
Diarrhoea	9	1	0	1	11 (31%)
Alopecia	6	4	0	0	10 (29%)
Fatigue	9	0	1	0	10 (29%)
Vomiting	9	1	0	0	10 (29%)
White blood count	2	1	4	1	8 (23%)
Anorexia	5	1	0	0	6 (17%)
Neutrophils	0	1	1	3	5 (14%)
Rhinitis	5	0	0	0	5 (14%)
Asthenia	3	0	1	0	4 (11%)
Platelets	2	2	0	0	4 (11%)

The table summarises all of the adverse events classified as possibly, probably or definitely related to the treatment with rubitecan and occurring in more than 10% of the cycles. No unexpected, cumulative or fatal toxicities were observed.

3.4. HPLC analysis

Peak area measurements of calibration standards were analysed by least squares linear regression analysis weighted by the reciprocal of the squared concentration. The equations of the calibration curves were used to calculate the concentration of the analytes in test and quality control samples. At least three quality control samples were processed and analysed in duplicate in every analytical run. At least 66% of the quality control samples were within $\pm 20\%$ of the nominal concentrations and at least 50% at each concentration level were within $\pm 20\%$ of the target.

3.5. Pharmacokinetics

Plasma samples were available from 17 patients (296 samples). 3 of these patients were found not evaluable for pharmacokinetic analysis. In 2 cases, the concentrations (fed and fasted) were below the detection limits for both rubitecan and 9-amino-camptothecin. One patient discontinued treatment during the pharmacokinetic sampling period. Therefore, 14 patients were evaluable for the statistical analysis of C_{max} and T_{max}. No significant differences were found between the two regimens due to the time period. No carry-over effects were detected, but there were subject within sequence effects, which reflected the expected intrapatient variability. Significant differences between fed and fasted were found for all of the tested pharmacokinetic parameters, as the 90% CIs were not within the predetermined interval of 0.8–1.25 (see Table 4; Fig. 3).

4. Discussion

The present study was performed to determine whether tumour responses can be achieved with rubitecan in non-pretreated patients with colorectal cancer, to further characterise the safety profile of the drug when given once daily for 5 consecutive days at a dose of 1.5–2.0 mg/m^2, and to assess the effect of food intake on the bioavailability of the oral topoisomerase I-inhibitor.

Table 3
Response assessment (WHO criteria)

	No. of patients (%)
Complete response	0
Partial response	0
No change/stable disease	3 (16)
Progressive disease	5 (26)
Early progressive disease	7 (37)
Non-evaluable[a]	4 (21)

WHO, World Health Organization.

[a] 2 patients received less than one treatment cycle, and in 2 other patients a full evaluation was not performed.

Table 4
Pharmacokinetic parameters for rubitecan after oral administration under fasted and fed conditions: intrapatient comparison (mean results± standard deviation)

	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-8\ h}$ (h×μg/l)	$AUC_{0-24\ h}$ (h×μg/l)
No. of evaluable pts	14	14	12	11
Condition				
Fasted	57.3±19.5	3.1±0.5	336.1±86.8	774.7±245.8
Fed	28.9±12.2	6.3±1.5	133.4±56.4	473.2±211.0
Two-tailed significance	$P<0.001$	$P<0.001$	$P<0.001$	$P=0.003$
90% Confidence Interval	1.74–2.36	0.44–0.57	1.99–3.47	1.34–2.20

T_{max}, time to maximal drug concentration; C_{max}, maximal drug concentration; AUC, area under the curve; pts, patients.

None of 15 fully evaluable patients had an objective response, and only 3 patients achieved a short-lasting disease stabilisation. This observation is very similar to the experience with another topoisomerase I-inhibiting drug. Topotecan was found to be inactive in colorectal cancer when given as a single agent, but appears to have good activity in other tumour types [14]. The only compound of this group with a relevant level of anti-tumour activity in colorectal cancer so far is irinotecan [15]. This topoisomerase I-inhibitor has become an integral component of the first- and second-line treatment of the most common gastrointestinal malignancy [16–18]. It appears though that the antineoplastic efficacy of topoisomerase I-inhibitors seems to be highly tumour-specific, at least in the clinical setting. Hopefully, rubitecan will be found to be valuable in other tumour types. Given the fact that the toxicity observed in our trial was very limited, it may be questioned whether or not the dose chosen was adequate in this study. It should be highlighted though that a number of responses have already been reported in diseases such as pancreatic, breast and ovarian cancer and other tumour types using a very similar dose and schedule.

In these and other clinical settings, the oral drug could be a very safe and well tolerated choice. The side-effects observed in our trial were only mild to moderate, with the exception of one case of diarrhoea. There were no unexpected, severe, life-threatening or cumulative events. Chemical cystitis, which has been reported as a potential side-effect of this agent, was effectively prevented by an increased oral fluid intake during the treatment period. The incidence of haemorrhagic cysti-

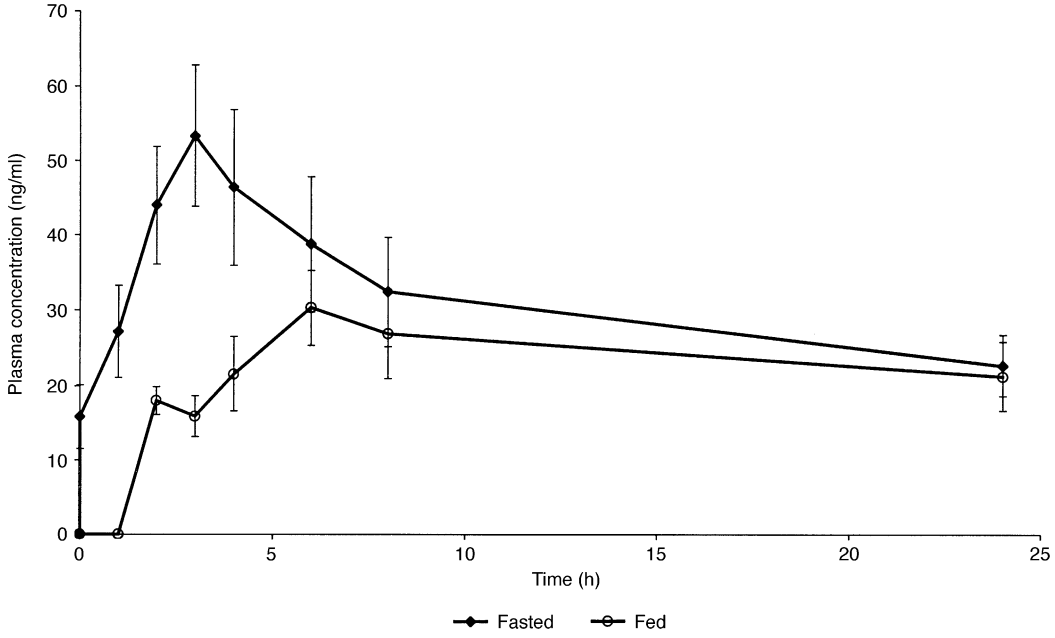

Fig. 3. Rubitecan plasma concentration after the administration of the drug under fasted or fed conditions ($n=14$ patients). Intrapatient variation of plasma concentrations of rubitecan (ng/ml) after a single oral administration of 1.5 mg/m^2 either after an overnight fast (full rhombus) or after a high calorie breakfast (empty circle). According to the pharmacokinetic data, food intake immediately prior to the drug administration leads to a significant decrease in the absorption rate and extent of absorption of rubitecan, which results in a decreased plasma concentrations as illustrated here.

tis in previous trials has ranged from 19 to 25% [7,19], while we observed nocturia/stranguria in only three treatment cycles which was limited to grade 1 CTC.

A safe and active oral palliation for gastrointestinal or other malignancies would certainly be of great value. The long-term enteral administration of topoisomerase I-inhibitors may have potential pharmacological advantages. *In vitro*- and *in vivo*-studies suggest that the prolonged exposure to this group of agents may be relevant to their antitumour activity [20]. Furthermore, oral treatment would be preferred by the majority of patients compared with an intravenous administration, as recently shown in a prospective study in the advanced colorectal cancer setting for a fluoropyrimidine drug [21]. For these, and other, reasons, various new formulations of topoisomerase I-inhibiting agents are currently under investigation, including oral rubitecan, oral topotecan, oral irinotecan and new intravenous liposomal camptothecin derivatives.

The validated HPLC assay used for the determination of total plasma concentrations of rubitecan revealed a major effect of food intake on the oral bioavailability of the agent. A high-calorie breakfast prior to the drug administration leads to a significant decrease in the absorption rate and extent of absorption of rubitecan, as indicated by the C_{max}, T_{max} and AUC.

Our findings clearly suggest that rubitecan should be taken under fasted conditions. Based on these observations, the current broad phase II evaluation of this promising agent in Europe is applying the appropriate fasting schedule, as supported by our pharmacokinetic data.

Acknowledgements

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