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Phase I and pharmacokinetic study of halofuginone, an oral quinazolinone derivative in patients with advanced solid tumours

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ABSTRACT

Purpose: Halofuginone (tempostatin™) is a synthetic derivative of a quinazolinone alkaloid showing anti-angiogenic, anti-metastatic and anti-proliferative effects in preclinical studies. The objectives of this phase I study were to assess the dose-limiting toxicities (DLTs), to determine the maximum tolerated dose (MTD) and to study the pharmacokinetics (PKs) of halofuginone when administered once or twice daily orally to patients with advanced solid tumours.

Methods: Patients were treated with escalating doses of halofuginone at doses ranging from 0.5 to 3.5 mg/day. For pharmacokinetic analysis plasma sampling was performed during the first and second course and assayed using a validated high-performance liquid chromatographic assay with mass spectrometric detection.

Results: Twenty-four patients received a total of 106 courses. The 'acute' MTD was reached at 3.5 mg/day, with nausea, vomiting, and fatigue as DLT. The recommended dose for chronic administration was defined as 0.5 mg/day with the requirement of 5HT3 antagonists to control nausea and vomiting considered as DLT. Several patients experienced bleeding complications on treatment with halofuginone in which a causal relationship could not be excluded. The PKs of halofuginone were linear over the dose range studied with a large interpatient variability.

Conclusions: In this study the DLT of halofuginone was nausea, vomiting, and fatigue. The recommended dose for phase II studies of halofuginone is 0.5 mg administered orally, once daily.

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1. Introduction

Recently the development of new anticancer agents has been accelerated by the unravelling of various processes involved in malignant transformation of cells and carcinogenesis, resulting in a large number of new anticancer agents targeting specifically one or more of these extracellular, transmembrane or intracellular processes. Examples of these new targets are matrix metalloproteinases (MMP) and angiogenesis. The invasive behaviour of neoplastic cells and their ability to metastasise to distant sites are multiple step processes that include detachment of the cells from the original tumour mass, attachment to the extra cellular matrix (ECM) binding sites, degradation of ECMs, and migration into surrounding tissues.[1] One of the rate-limiting steps in the metastatic cascade is the activity of MMPs degrading a variety of ECM proteins. MMP-2 plays a critical role in tumour cell invasion and metastasis. Halofuginone hydrobromide (*trans*-7-bromo-6-chloro-3-[3-(3-hydroxy-2-piperidyl)-2-oxopropyl]-4(3H)-quinazolinone hydrobromide) salt (Tempostatin™) is a derivative of febrifugine, an alkaloid originally isolated from the plant, *Dichroa febrifuga* (Fig. 1). It is a drug that was originally developed and approved by the US Food and Drug Administration in the early 1980s for the veterinary market for the prevention of coccidiosis in growing chickens and turkeys for human consumption.[2] Halofuginone hydrobromide is a small molecule that might affect tumour growth via several mechanisms. It acts as a potent reversible inhibitor of collagen type I synthesis at the transcriptional level[3–6] and suppresses ECM deposition.[7] Halofuginone was also shown to inhibit TGF-β-stimulated collagen α1 (I) synthesis by human skin fibroblasts.[3,8] Collagen type I is critically involved in complex processes related to tissue remodelling, cell adhesion, cell proliferation, and cell migration, which, in turn, are critical prerequisites for processes such as angiogenesis, primary and metastatic tumour growth. Moreover, exposure to halofuginone was found to inhibit deposition of ECM by vascular smooth muscle and kidney mesangial cells.[7,9] In addition, halofuginone was found to inhibit the expression of the MMP-2 gene at concentrations as low as 50 ng/ml based on an effect on the activity of the MMP-2 promotor resulting in a potent anti-metastatic activity *in vitro*.[7,10] The anti-angiogenetic properties of halofuginone were demonstrated by exposing rat aortic rings embedded in a collagen gel to halofuginone demonstrating that halofuginone inhibited the invasion of endothelial cells into the collagen gel and their subsequent alignment and branching into microvessels.[7,11] Furthermore, halofuginone also inhibited bFGF-induced angiogenesis in the mouse corneal micro pocket assay.[7]

In vitro, halofuginone exerted anti-tumour activity against a variety of murine and human tumour cell lines including rapidly proliferating murine B-lymphoma and human myeloid leukaemia cells, melanoma, sarcoma, bladder, prostate and breast carcinoma cell lines. *In vivo*, halofuginone significantly inhibited the progression of human melanoma, sarcoma, brain tumour, bladder, breast, prostate cancer xenograft both after intraperitoneal and oral administration.[10–15]

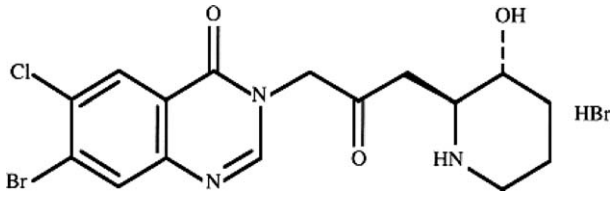

Fig. 1 – Chemical structure of halofuginone.

In mice and dogs, oral halofuginone at dose levels up to 0.35 and 0.142 mg/kg/day, respectively, for 4–26 weeks was well tolerated resulting in a decrease in haemoglobin, leukocytes and mean cell blood volume and increase in urea and cholesterol and a decrease in food consumption. Mortality was observed in mice at 6 mg/kg/day base occurring within 5 days of treatment. Pre-mortem signs included piloerection, ptosis, irregular respiration and lethargy. In dogs administration of 0.075 mg/kg, bid for a period of 14 days, has shown vomiting at 30–60 min after dosing. During studies performed in new born calves treated orally with halofuginone diarrhea, inflammation and congestion of the gastrointestinal tract was noted, which could be alleviated by administering the drug with food. Furthermore, no cardiovascular effects were observed after oral administration of halofuginone to conscious beagle dogs. Long-term studies in mice and rats did not reveal carcinogenic or mutagenic potential of halofuginone (data on file).

The pharmacokinetics of halofuginone have been studied in several animal species as well as in human volunteers. After single intravenous and subcutaneous administration, halofuginone was rapidly distributed out of the plasma compartment and into the tissues resulting in a large volume of distribution. The oral availability of halofuginone in rats and pigs was 51% and 72%, respectively. Disposition studies in animals revealed that 60% of the oral dose was eliminated unchanged in the faeces. Renal clearance was limited to 7.5–16.7% of total body clearance and only 15–16% of the parent compound was excreted in the urine within 48 h after oral administration. The unchanged drug was the major component in plasma, no major metabolites were identified. Plasma-half life varied between 5 and 17 h.[16] Repeated daily oral doses in pigs did not reveal apparent drug accumulation.

In a double-blind, escalating single oral dose study of halofuginone in healthy male volunteers, no serious adverse events were reported at doses of 0.07 and 0.5 mg. With dose increments of 1.5, 2 and 2.5 mg, mild to moderate episodes of vomiting and nausea were reported, but these were of short duration and resolved spontaneously. Multiple daily dosing of halofuginone reduced the incidence of vomiting (data on file).

The purposes of the present phase I study were to determine the MTD of halofuginone administered orally once or twice daily, to establish the dose limiting and other toxic effects, to describe the PKs of halofuginone with respect to interpatient and intrapatient variation, to document any anti-tumour effects and to establish a dose suitable for further phase II evaluation of activity of the compound.

2. Patients and methods

2.1. *Patient selection*

Patients with a cytologically or histologically confirmed diagnosis of a malignant solid tumour refractory to standard forms of therapy were eligible for this study provided that

they met the following criteria: age $\geqslant$18 years; WHO performance status $\leqslant$2; estimated life expectancy $\geqslant$12 weeks; no previous chemotherapy for at least 4 weeks; no radiotherapy for at least 6 weeks; no significant stomach or small intestine disease that might influence the absorption of the drug; and adequate haematopoietic (haemoglobin $\geqslant$6.2 mmol/L, absolute peripheral granulocyte count $\geqslant 1.5 \times 10^9$/L, WBC $\geqslant 3.0 \times 10^9$/L and platelet count $\geqslant 100 \times 10^9$/L), hepatic (bilirubin $\leqslant$1.5 times the upper normal limit, and serum aspartate aminotransferase and alanine aminotransferase $\leqslant$2.5 times the upper normal limit) and renal (serum creatinine concentration $\leqslant$1.5 times the upper normal limit) functions. Patients with symptomatic brain or leptomeningeal metastases were excluded. All patients gave written informed consent before study entry. The study was approved by the Institutions Medical Ethic Committees.

2.2. *Treatment and dose escalation*

Halofuginone was supplied by Collgard Biopharmaceuticals Ltd. (Tel-Aviv, Israel) as round tablets of about 200 mg comprising 0.5, 2.5 or 5.0 mg of active drug and aerosil, crospovidone, lactose, talc, avicel and Mg stearate. The tablets were stored at 2–8 °C. Tablets were taken once a day during breakfast or twice daily during breakfast and dinner. The daily dose of halofuginone was provided in separate boxes, with each daily dosing clearly identifiable by the patient. Patients were instructed to record their daily amount of tablets taken, the time of administration, and the timing in relation to breakfast or dinner. Compliance with the scheduled treatment was assessed at the end of each cycle, by counting the used and returned capsules of halofuginone in relation to the record kept by the patient for the given cycle. A cycle was defined as a period of 2 weeks. With the exception of the first cycle, during which patients were hospitalised for pharmacokinetic sampling for 3 days, patients were treated on an outpatients basis.

The starting dose of halofuginone was 1 mg/day, 1/3 of the no observed adverse effect level and 1/10 of the maximum tolerated dose (MTD) in rat. The total dose prescribed was rounded to the nearest 0.5 mg. During the first cycle halofuginone was administered on day 1 followed by 2 days with no medication administration in order to describe the full kinetic profile of the drug. Daily treatment was resumed on day 4. Initially, only one patient per dose level had to be treated. In case a patient at a given dose level experienced grade 3 haematological toxicity and/or non-haematological toxicity $\geqslant$grade 2 during the first cycle, the dose level had to be expanded to three patients. Dose escalations were based on the prior dose level toxicity and pharmacological data allowing a dose escalation up to 100% (which was determined by the worst significant toxicity). Once $\geqslant$grade 2 non-haematological toxicity or $\geqslant$grade 3 haematological toxicity was observed in one patient, further dose escalation would follow a modified Fibonacci scheme. As soon as a DLT was observed the CRM model provided dose recommendations. Because of the mechanism of action of the drug a chronic dosing regimen would be preferred for further development of the drug, therefore it was decided to determine two MTDs. First an 'acute' MTD during the first 2 weeks of treatment, and second, a 'chronic' MTD over the first 8 weeks of treatment. The 'acute' MTD was defined as the dose level that induced DLT in 20% of patients. DLTs for determining the 'acute' MTD were defined as grade 4 granulocytopenia for 7 or more days, febrile neutropenia, platelets $<25.0 \times 10^9$/L or $<50.0 \times 10^9$/L and complicated by bleeding and/or non-haematologic toxicity $\geqslant$grade 3. Nausea and vomiting subsequently responding to anti-emetic therapy were excluded from being an acute DLT. For the 'chronic' MTD any haematologic toxicity $\geqslant$grade 3 and/or any toxicity resulting in omission of more than 10% of the intended dose and/or nausea/vomiting requiring the administration of 5HT3 antagonist were also considered as a DLT. The dose recommended for further investigation was defined as the dose that could be administered for 8 weeks with a 90% dose-intensity in 80% of the patients without causing any toxicity >grade 2.

If a patient encountered DLT, the treatment of halofuginone was interrupted until resolution of toxicity to $\leqslant$grade 1, with a maximum of 1 week. If after re-introduction of the drug the toxicity re-occurred, halofuginone was decreased with one dose level at re-treatment. In case toxicity persisted after 1 week of treatment interruption but resolved during the second week, halofuginone was decreased with one dose level at re-treatment. If toxicity had not resolved to $\leqslant$grade 1 after 2 weeks of interruption, patients would go off study. Toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria, version 2.0.

2.3. *Treatment assessment*

Before treatment a complete medical history was recorded and a physical examination performed. A complete CBC including white blood cell differential, and serum biochemistry, which involved sodium, potassium, calcium, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine phosphokinase, were performed, as were urinalysis, pregnancy test, relevant tumour markers, electrocardiogram and a chest X-ray. Weekly evaluations included history, physical examination, toxicity assessment according to the CTC criteria, CBC and serum chemistries. Tumour evaluation was performed every 8 weeks according to the Response Evaluation Criteria in Solid Tumours (RECIST).[17] Patients were taken off protocol at the onset of disease progression.

2.4. *Sample collection and drug analysis*

For pharmacokinetic analysis, ten blood samples (~5 mL each) were obtained from an indwelling intravenous canula and collected in vials containing lithium heparin as anticoagulant. The samples were taken immediately before dosing on day 1 and 1, 2, 4, 8, 24, 48 and 72 h after administration of the drug. On day 15 and 29 samples were taken prior to dosing to measure the accumulation of halofuginone.

All samples were cooled on ice and centrifuged immediately after sampling at 1500*g* for 10–15 min at 4 °C and the plasma was stored at −20 °C or lower in polypropylene tubes in the dark until analysis. A total of four urine samples were also collected over a 48-h period; 0–6 , 6–12 , 12–24 and 24–48 h post-dosing on day 1 of the first cycle only. Of the total amount

collected, a measured quantity of 1 mL in duplicate was drawn and stored at −20 °C or below until analysis. Concentrations of halofuginone in plasma were determined according to a validated liquid chromatography–mass spectrometry detection method with liquid–liquid extraction at CEPHAC (CEPHAC, 90 avenue des Hauts de la Chaume, B.P. 28, 86281 St. Benont Cedex, France).The lower limits of quantitation were 0.10 and 0.50 ng/mL for plasma and urine samples respectively.

2.5. *Pharmacokinetic data analysis*

The terminal disposition half-life [$T_{1/2}(z)$] of halofuginone was calculated as $\ln 2/k$, where k is the terminal elimination rate constant (expressed in h^{-1}). The peak plasma concentrations (C_{max}) and the time to peak plasma concentration (T_{max}) were determined graphically from the experimental values. The area under the plasma concentration–time curve (AUC) of halofuginone were estimated using the experimental values (trapezoidal rule) with extrapolation to infinity ($AUC_{0-\infty}$) using the terminal elimination rate constant, defined as the slope of final 3–4 data points of the log-linear concentration–time plot. The total body clearance (CL) was calculated as the ratio between the administered dose and the $AUC_{0-\infty}$. The extent of accumulation (R_0) was calculated as the ratio between the plasma concentration 24 h after dosing on day 15 and day 1. The fraction of the administered dose (F_e) excreted in the urine and the cumulative amount of halofuginone excreted in the urine (A_e) was determined. PK data analysis was carried out using a non-compartmental analysis approach with the aid of WinNonlin Version 3.0 (Pharsight, 1999).

2.6. *Statistical analysis*

All pharmacokinetic data are presented as mean values and coefficient of variability. The effect of drug dose on clearance, volume of distribution and terminal disposition half-life was analysed using a Kruskal–Wallis multiple comparison test. The level of significance was set at $P = 0.05$.

3. Results

Between August 2001 and February 2004 25 patients, whose main characteristics are listed in Table 1, were enrolled onto the study at two centres. All patients were eligible apart from one who had undergone a partial esophagectomy and gastrectomy prior to study entry and did not fulfill the entry criteria. The last patient entered did not receive treatment. The majority of the patients were either asymptomatic or had only mild symptoms at study entry. Patients were pre-treated with median three prior chemotherapy regimens (range 1–5). The total number of assessable cycles was 106. The median number of cycles per patient was 4 (range 0–15). Dose levels studied were 1, 2, 3.5, 0.5 mg/day and 0.5 mg twice daily administered orally on a continuous basis, with cycles defined as a treatment period of 14 days (Table 2).

Table 1 – Patient characteristics

Characteristic	Number of patients
Number of entered	25
Number of assessable	24
Age, years	
Median	58
Range	21–75
Sex	
Female	9
Male	15
Performance status	
WHO 0	11
WHO 1	12
WHO 2	1
Tumour type	
Melanoma	6
Renal	4
NSCLC	4
Sarcoma	3
Miscellaneous	7
Previous treatment	
Chemotherapy	22
Radiation	12

Table 2 – Dose levels studied

Dose (mg/day)	Number of patients	Number of cycles/ range per patient
1	1	4
2	6	19/1–4
3.5	3	16/2–12
1	3	13/4–5
0.5	5	33/4–15
0.5 bid	7	21/0–5

3.1. *Tolerability*

According to protocol in the first part of the study the 'acute' MTD was defined during the first 2 weeks of treatment. No DLT was observed at 1 mg/day. Since grade 2 nausea was observed at 2 mg/day the cohort was expanded to three patients. All three patients experienced grade 2 nausea/vomiting. Therefore another three patients were entered at the same dose with the introduction of prophylactic anti-emetics. No DLTs were encountered at the 2 mg/day dose level. However at 3.5 mg/day two patients experienced DLT despite prophylactic anti-emetics. One patient had grade 3 nausea, accompanied by grade 2 vomiting, grade 2 dehydration and grade 3 fatigue. The other patient experienced fatigue grade 3, hyponatremia grade 4 and nausea grade 2. It was concluded that the 'acute' MTD was exceeded and the first phase of the study completed. In order to define the 'chronic' MTD the dose was de-escalated. Since according to the definition of 'chronic' DLT also the requirement of 5HT3 antagonists to control nausea and vomiting was considered a DLT, and at the 2 mg dose level 5 out of 6 patients had used 5HT3 antagonists, the 1 mg dose level was expanded. One patient erroneously only used 0.5 mg/day and had to be replaced. Of the three evaluable patients, one patient experienced a DLT requiring the use of 5HT3 antagonists to control nausea and vomiting. According to the protocol the next lower dose level of 0.5 mg/day was subsequently

explored. Five patients were treated at this dose level and none experienced a DLT. Based on these data it was decided to study the 0.5 mg twice daily dose level. Two out of six patients at this dose level had nausea/vomiting grade 2 requiring the use of 5HT3 antagonists, defining the recommended dose of halofuginone for further studies at 0.5 mg once daily. Based on these findings the seventh patient, who had already given his informed consent, was withdrawn from the study and did not start treatment.

Overall halofuginone predominantly caused gastro-intestinal toxicity consisting of nausea and vomiting chronologically related to the administration of the drug (Tables 3 and 4). Most patients experienced nausea and vomiting within 30 min to 1 h after ingestion of the drug. Only at the lowest dose level did no patients require 5HT3 antagonist to control nausea and vomiting. However, even at this dose level of 0.5 mg/day three out of five patients used domperidon or metoclopramide as anti-emetics.

Fatigue was the second most common side-effect. However, this might partially be related to the disease.

No haematological toxicity was observed.

Seven patients experienced bleeding complications during treatment with halofuginone, divided over all dose levels. At the 2 mg/day dose level one patient, with extensively metastatic melanoma, developed a bleeding in an, until then, unknown brain metastasis during cycle 3. A second patient at this dose level experienced a gastric bleeding with gastroscopy showing a benign ulcer during cycle 4. One patient with a carcinoma of unknown primary treated at 1 mg/day dose level had epistaxis. At the 0.5 mg/day dose level a patient with metastatic renal cancer died due to a bleeding in an unknown brain metastasis. A second patient at this dose level showed an enlargement of an old haematoma during the first treatment cycle. At the 0.5 mg twice daily dose level two patients also had a bleeding complication; one patient with a metastatic rectal cancer died suddenly during cycle 5 due to massive hepatic bleeding of liver metastases. The last patient with a metastatic melanoma experienced intestinal bleeding requiring multiple transfusions during cycles 2–4. No changes in thrombocyte counts, nor clotting parameters, were recorded in any of the patients. This number of bleeding complications is reason for sincere concern. Although it might be a coincidence in a patient population known to be prone to developing bleedings at metastatic sites, the total number encountered could point to a possible relationship with the administration of the study drug. Further drug administration therefore requires relevant measurements to monitor this possible side-effect.

3.2. *Antitumour activity*

No objective responses were observed. Six patients experienced stabilisation of their disease (sarcoma, melanoma, non-small cell and small cell lung, rectal and renal cancer) for a median duration of 11 weeks (range 8–30 weeks).

3.3. *Pharmacokinetics*

Full kinetic data were obtained from 24 patients following the administration of halofuginone.

The plasma concentration–time profiles of halofuginone were similar for all patients studied, as shown in Fig. 2. The absorption of halofuginone after oral drug administration was associated with maximum peak drug levels at 3.4 ± 4.8 h. Inspection of the scatterplots of dose versus $AUC_{0-\infty}$ (Fig. 3A) and C_{max} (Fig. 3B) for halofuginone revealed an increase in both parameters with the dose level administered.

The long half-life resulted in accumulation of halofuginone, such that exposure was two- to three-fold higher by day 15 of dosing.

Table 3 – Toxicity in the first cycle according to NCI-CTC version 2.0

Dose (mg/day)	No. pts	WBC				ANC				Plt				Nausea			Vomiting				Fatigue				DLT
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	1	2	3	4	1	2	3	4	
1	4	–	–	–	–	–	–	–	–	1	–	–	–	3	–	–	2	–	–	–	3	–	–	–	1*
2	6	–	–	–	–	–	–	–	–	–	–	–	–	1	4	–	2	3	–	–	2	3	–	–	–
3.5	3	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	1	1	–	–	–	–	2	–	2
0.5	5	1	–	–	–	–	–	–	–	–	–	–	–	1	–	–	2	–	–	–	1	–	–	–	–
0.5 × 2	7	–	–	–	–	–	–	–	–	–	–	–	–	2	–	–	1	1	–	–	3	–	–	–	2*

Abbreviations. No. pts, number of patients; Plt, platelets; DLT, dose limiting toxicity; *, DLT for chronic administration.

Table 4 – Worse toxicity per patient for all cycles according to NCI-CTC version 2.0

Dose (mg/day)	No. pts/cycles	WBC				ANC				Plt				Nausea			Vomiting				Fatigue			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	1	2	3	4	1	2	3	4
1	4/17	–	–	–	–	–	–	–	–	1	–	–	–	2	2	–	3	1	–	–	–	4	–	–
2	6/19	–	–	–	–	–	–	–	–	–	–	–	–	2	4	–	2	3	–	–	2	3	–	–
3.5	3/16	–	–	–	–	–	–	–	–	–	–	–	–	–	1	1	2	1	–	–	–	1	2	–
0.5	5/33	1	–	–	–	1	–	–	–	–	–	–	–	2	1	–	3	–	–	–	1	–	–	–
0.5 × 2	7/21	–	–	–	–	–	–	–	–	–	–	–	–	4	1	–	2	2	–	–	3	–	–	–

Abbreviations. No. pts, number of patients; Plt, platelets.

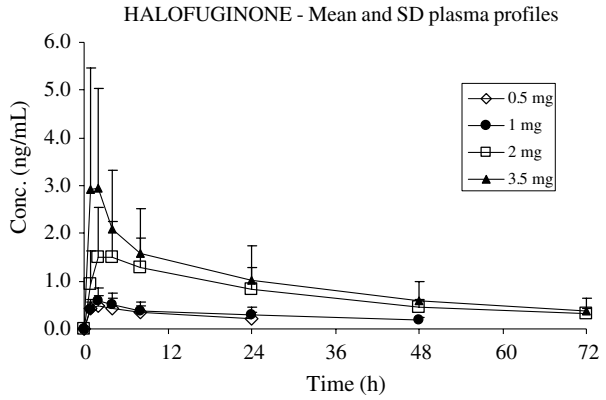

Fig. 2 – Mean (+SD) plasma concentration–time profiles observed after first administration of halofuginone at different dosages.

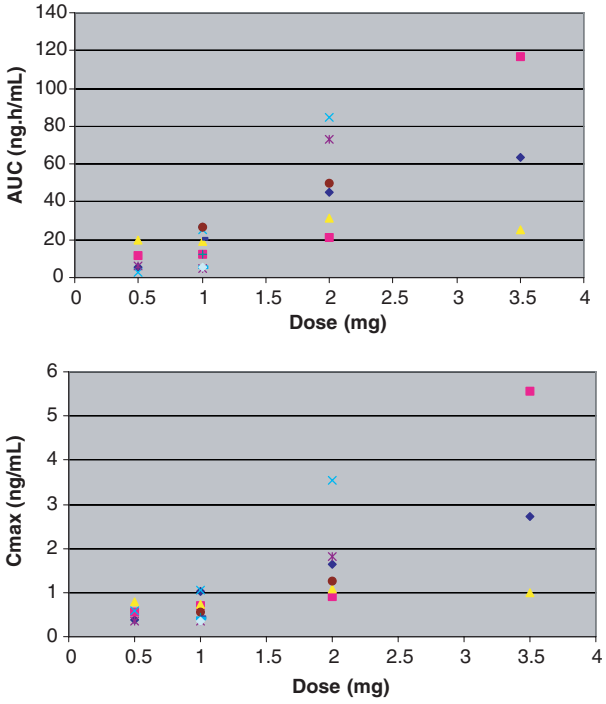

Fig. 3 – Relationship between AUC (A) and C_{max} (B) on day 1 of the first cycle of halofuginone as a function of dose administered per day.

The interpatient variability in the observed pharmacokinetics was large, with coefficients of variation in AUC values as high as 74%. Elimination of halofuginone from the central plasma compartment was characterised by decay in an apparent tri-exponential manner based on conventional compartmental modelling using weighted least-squares analysis with a weighting factor of 1/Y. The estimated terminal elimination half-life was relatively constant in all subjects, exhibiting mean values of 28.3 ± 12.9 h, and was not dependent on the dose of halofuginone. The percentage of extrapolated $AUC_{0-\infty}$ was more than 20% in most of the subjects, indicating that the $AUC_{0-\infty}$ values should be considered with caution.

The total plasma clearance and estimated terminal half-life of halofuginone were not different between the various dose levels suggesting a linear pharmacokinetic behaviour. The mean pharmacokinetic parameters determined using a non-compartmental analysis are listed in Table 5. Only 4–12% of the administered dose of halofuginone was excreted unchanged in the urine mainly in the first 24 h after drug administration.

4. Discussion

Based on their mechanism of action, agents like halofuginone are thought to be most effective when administered for prolonged periods of time. The prolonged administration of these agents has implications for the side-effects to be tolerated by the patient and therefore the definition of DLT. In the present study it was decided that for long term use the requirement of 5HT3 antagonists to control nausea and vomiting would be considered a DLT, also based on the side-effects i.e. constipation, known to occur with prolonged administration of this class of anti-emetics. Indeed, nausea and vomiting, together with fatigue were the dose limiting side-effects of halofuginone. Both nausea and vomiting were chronologically related to the administration of halofuginone, occurring in most patients within 30 min to 1 h after ingestion of the drug. Four patients (16%) stopped treatment because of the experienced gastro-intestinal side-effects. Even at the lower dose levels most patients required the introduction of 5HT3 antagonists to control nausea and vomiting, defining 0.5 mg halofuginone administered once daily as the recommended dose for further studies.

It was noted that seven out of 24 patients developed bleeding complications during the study, especially at metastatic sites. While for each case considered separately a causal relationship with the study drug could not be proven, further studies are needed to exclude any correlation and caution will have to be taken during future studies incorporating the evaluation of this toxicity. In preclinical studies halofuginone inhibited the migration of endothelial cells and prevented their organisation into networks of branching and anastomising capillary-like tubes. Thrombo-embolic or bleeding complications were not observed in *in vivo* studies.

No objective tumour responses were observed during the study. In two patients, prolonged disease stabilisation was observed for 24 and 30 weeks. The time to progression on their previous chemotherapy regimens was 28 and 29 weeks, respectively. It has been postulated that activity of non-cytotoxic anticancer agents might be evaluated by comparing sequentially measured paired failure times within each treated patient.[18] A cytostatic agent should be considered effective if the time to progression after treatment with the cytostatic agent compared to the time to progression on their most recent prior therapy has a ratio greater than 1.33. When this assumption is applied to our present study, no strong evidence for anti-tumour activity could be objectivated.

Pharmacokinetics revealed a long half life of halofuginone of approximately 30 h. The long half-life resulted in accumulation of halofuginone. However, the increase in exposure by day 15 was not accompanied by worsening of the gastro-intestinal side-effects. Despite the long half life, twice daily dosing of halofuginone was studied, since it was hypothesised that nausea might be related to peak plasma concentrations

Table 5 – Summary of the pharmacokinetics of halofuginone during the first treatment course

Dose level (mg/day)	*n*		AUC_{0-t} (ng h/mL)	$AUC_{0-\infty}$ (ng h/mL)	C_{max} (ng/mL)	T_{max} (h)	$T_{1/2}$ (h)	R_0
1	4	Mean	20.88	27.96	0.88	2.0	31.5	3.37
		CV%	32	31	21	0	42	34
2	6	Mean	50.85	67.97	1.7	3.2	37.0	2.46
		CV%	47	40	56	82	46	27
3.5	3	Mean	37.76	85.66	3.09	1.4	30.8	6.58
		CV%	65	71	75	47.	12	120
0.5	5	Mean	9.13	12.58	0.54	2.8	17.2	2.65
		CV%	74	61	34	105	54	42
2 × 0.5	6	Mean	12.5	15.2	0.42	5.8	25.0	3.72
		CV%	73	51	19	154	27	25

Abbreviations. *n*, number of patients; CV, coefficient of variation; AUC, area under the concentration–time curve; C_{max}, peak plasma level; T_{max}, time to maximal concentration; $T_{1/2}$, terminal elimination half-life; R_0, ratio of accumulation.

of the drug. Yet, it turned out that it was not feasible to escalate the dose to 0.5 mg twice daily due to encountered side-effects. The observed interpatient variability in AUC for halofuginone was large with a coefficient of variability of 74%. This is, however, in the range observed for other oral anti-tumour agents. The AUC reached at the recommended dose of 0.5 mg/day is within the range in which anti-tumour efficacy was observed in preclinical studies.

In conclusion, the predominant side-effect of the administration of oral halofuginone constituted of nausea defining the recommended dose for further studies at 0.5 mg/day. The high number of observed bleeding events necessitate caution in subsequent clinical studies. Presently, halofuginone has entered phase 2 studies, among others in bladder cancer.

Conflict of interest statement

None declared.

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