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Phase I clinical and pharmacological evaluation of the multi-tyrosine kinase inhibitor SU006668 by chronic oral dosing

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ABSTRACT

SU006668, an oral inhibitor of vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR), was administered in fed conditions to 24 patients with advanced solid cancer at 100, 200 and 300 mg/m^2 b.i.d. Dose escalation was discontinued because the maximum tolerated dose was defined at 400 mg/m^2 b.i.d in a concomitant trial. The drug was generally well tolerated although two patients presented possibly drug-related dose-limiting toxicities (pericardial effusion and thrombocytopenia). SU006668 had a non-linear pharmacokinetic profile characterized by AUC and Cmax decreasing from day 1 to day 28 in all patients at all tested doses; a lower apparent bioavailability on day 28 compared to day 1; and a significant concomitant increase of the urinary metabolites. These findings are in agreement with the presence of saturable absorption and metabolic induction. The peculiar pharmacokinetics and >99% protein binding discouraged further clinical development of oral SU006668 in humans.

1. Introduction

SU006668 is a selective inhibitor of the receptors for vascular endothelial growth factor (VEGF, Flk-1/KDR), platelet-derived growth factor (PDGFRβ) and fibroblast growth factor (FGFR1) (Fig. 1) [1]. SU006668 inhibits the growth of tumour cell lines in vitro (A431 non-small cell lung cancer, colon 205 and H460 lung) [1,3], and has significant antitumour activity in xenografts after daily oral (PO) treatment [2].

In rodents and dogs the pharmacokinetic disposition of SU006668 was linear [3]. Furthermore the plasma levels after oral administration were above the IC50 for VEGF and FGF receptors. Finally, the oral bioavailability in dogs was 10%, but increased up to 56% when the drug was given to fed instead of fasting animals [3]. The drug, as shown by pharmacokinetic investigations of the radiolabeled compound in rats, is mainly excreted through the bile [3].

Based on the appealing pharmacology described above, the oral formulation was tested in Phase I studies to define the optimal schedule of treatment and the effect of food on absorption. Data in humans confirmed that higher areas under the plasma concentration-time curve (AUC) were

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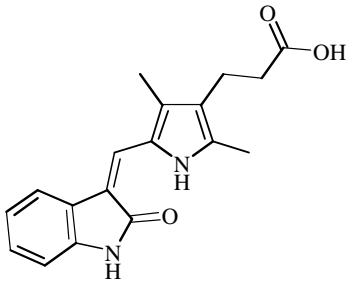

Fig. 1 – Chemical structure of SU006668.

achieved in fed conditions, and that such increased exposure was associated with a lower tolerability of more fractionated daily doses. Fatigue, thoracic pain with serositis, nausea, diarrhoea and gastro-intestinal discomfort were the main toxicities after b.i.d. or t.i.d. continuous dosing at the maximum tolerated doses (MTD) of 800 and 400 mg/m^2, respectively [4,5]. Meanwhile, the Phase I study of the intravenous drug was prematurely terminated because of chemical instability of the formulation [3].

The present study was set up to define the MTD of oral SU006668 given b.i.d. to fed patients, and to characterize the pharmacokinetic (PK) profile after chronic administration. In addition, validation of pharmacodynamic (PD) markers and PD correlation were investigated. Here we report the results of the clinical assessment and of the PK evaluation, while those of the PD study will be reported separately.

2. Patients and methods

The study was conducted in three centres with the approval of the relevant Independent Ethics Committees.

2.1. *Eligibility*

The following criteria were applied: histologically confirmed diagnosis of solid tumour insensible to conventional treatments; age > 18 yr; a maximum of three prior chemotherapies; at least one measurable or evaluable lesion; and normal haematological, renal, liver (AST and/or ALT $\leqslant 3 \times$ Upper Normal Limit [UNL] in case of liver metastases), and cardiac function (left ventricular ejection fraction [LVEF] $\geqslant$ 55%). Brain metastases; non insulin-dependent diabetes with severe peripheral vascular disease or diabetic ulcers or insulin-dependency; physical or electrocardiogram (ECG) evidence of coronary artery disease; clinical manifestations of cardiac ischemia in the preceding 6 months; and malabsorption were criteria for exclusion. All patients signed informed consent.

2.2. *Drug formulation and administration*

Oral SU006668 was supplied as 150 mg capsules to be stored at 15–30 °C. The total daily dose was administered in two fractions, each to be taken within 1 h of meals.

SU006668 for injection was supplied as a 50 ml vials containing 450 mg/vial of SU006668 and 450 mg/vial of mannitol. Each vial was reconstituted with 30 ml of diluent to a final concentration of 15 mg/ml and further diluted with 0.9% sodium chloride to 4 mg/ml. The final solution had a pH between 8.07 and 8.26 and was stable for 24 h at room temperature. The drug was infused over 30 min.

2.3. *Study design*

The study design is described in Fig. 2. On days of PK sampling, a standard breakfast with defined calories and fat content was provided. SU006668 was given continuously PO b.i.d. in fed conditions. To assess bioavailability, an intravenous (iv) test dose of 100 mg/m^2 was given 7 days (−7) before starting the oral treatment and a PK profile was assessed for 24 h. A full PK study was performed on the first day of PO dosing (day 1) and was repeated after 28 days of oral dosing, after which a second iv dose of 100 mg/m^2 was administered on day 29 (see Fig. 2). PO treatment b.i.d. in fed conditions was restarted on day 30; patients in response or with stable disease could continue therapy.

The effects of SU006668 on angiogenesis and related signal transduction pathways were analyzed in skin biopsies (Fig. 2) according to previously described procedures [6–8]. Data from this part of the investigation will be published elsewhere.

Dose escalation started at 100 mg/m^2 b.i.d., a dose that was already known to be safe after dosing for 56 days (personal communication). Subsequent dose levels were 200 and 400 mg/m^2, the latter with the possibility of adjustment according to the results of the other ongoing Phase I trials. The MTD was defined as the dose at which $\geqslant$2 of 6 patients experienced a dose-limiting toxicity (DLT). At least 3 patients had to be treated per dose level, 6 in case of toxicity, the second and the third after the first had completed at least one week of therapy. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria scale (NCI-CTC V2.0, 1999). DLT was defined as any one of the following: drug-related death; neutropenia/leukopenia grade (G) 4; thrombocytopenia and renal toxicity G $\geqslant$ 2; anaemia, pain of any origin, fatigue, and hepatic, cardiac or any other toxicity G $\geqslant$ 3; any other toxicity of lower grade requiring treatment discontinuation not recovered to G $\leqslant$ 1 within 2 weeks. During each cycle, in case of G $\geqslant$ 2 toxicity (except for nausea/vomiting) treatment could be discontinued for a maximum of 2 weeks to allow for recovery to G $\leqslant$ 1 and thereafter resumed at the same dose level. In case of DLT, treatment could be resumed at the immediately preceding dose level.

Pre-trial screening consisted of medical history and tumour assessment within 4 weeks prior to first iv test dose; complete physical examination, chemistry (total bilirubin, ALT, AST, creatinine kinase, LDH, alkaline phosphatase, total protein and albumin, electrolytes, calcium), complete blood cell count (CBC) with differential, urinalysis, 12-lead ECG, LVEF measurement (by ultrasound or MUGA scan) and pregnancy test within 7 days prior to first iv dose. Chemistry and haematology were repeated weekly during the first cycle of therapy and then on days 8 and 28. At the end of each cycle, complete physical examination and 12-lead ECG were performed. LVEF measurement was reassessed after cycle 1 and thereafter only if clinically indicated.

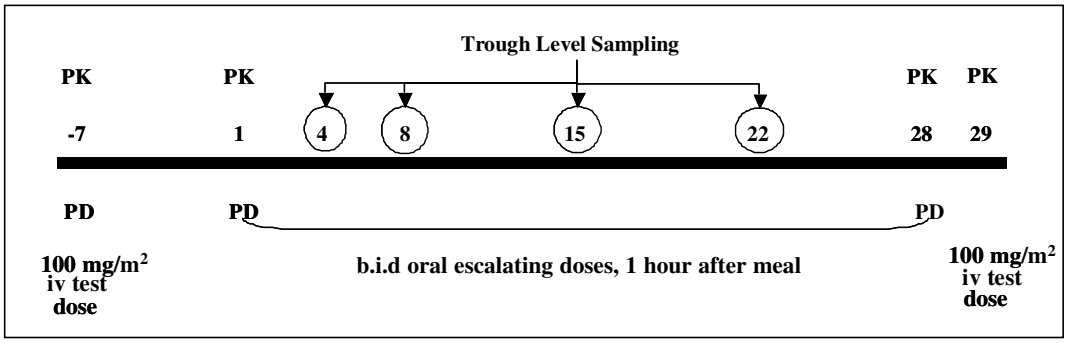

Fig. 2 – Study design. SU006668 was orally administered b.i.d., every 12 h, in fed conditions from day 1 to day 28. On days −7 and 29 a dose of 100 mg/m^2 was infused over 30 min. Pharmacokinetic assessment (PK) was done on days −7, 1, 28 and 29 and additional samples were collected on days 4, 8, 15 and 22. Coupled skin biopsies were taken on days −7 and 1, and again on days 29 and 35 for pharmacodynamic evaluation (PD).

Tumour assessment was performed according to RECIST criteria [9] within 4 weeks prior to study entry, at the end of cycle 1 (after 28 days of PO treatment) and then every two cycles.

2.4. *Pharmacokinetic analysis*

SU006668 was measured in plasma and urine after intravenous dosing on day 7 and 29 and after oral dosing on days 1 and 28 of cycle 1. Additional plasma samples were taken on days 4, 8, 15 and 22 of cycle 1 for trough level assessments. In the expansion phase of the study, the intravenous dosing was not performed and PK assessment of SU00668 was done only on days 1 and 28 of cycle 1.

Blood samples (2 ml) were collected from a vein in the opposite arm of intravenous infusion: before treatment, immediately before the end of infusion, at 5, 10, 20, 30, 60 min and at 2, 3, 4, 6, 8, 12, 24 h after the end of infusion. PK after oral dosing was assessed on blood samples collected before and at 30 and 60 min and at 2, 3, 4, 6, 8, 12 (before the second dose) and 24 h after the first dose.

Blood was transferred into glass tubes containing lithium heparin pre-refrigerated in ice and protected from light. Plasma was separated by centrifugation at 2000*g* for 10 min at 4 °C, transferred into polypropylene vials and stored at −20 °C until analysis. Urines were collected on days −7, 1, 28, 29 as 12 h aliquots in ambered containers kept at 4 °C. About 2 ml of Tris buffer solution, 3.3 M, pH 8, was added to each 100 ml of urine to dissolve precipitates. A 20 ml aliquot of each urine fraction was stored at −20 °C.

2.5. *SU006668 plasma level measurements*

A high performance liquid chromatography (HPLC) method was developed and validated for the quantitation of SU006668 in human plasma in a range of 10–25,000 ng/ml.

After addition of SU009905 as internal standard, 0.1 ml of plasma was extracted on line on a RP-18 solid-phase extraction cartridge. After washing, the cartridge was automatically moved on line with the chromatographic system. Chromatographic separation was achieved on an ion exchange analytical column LC-ABZplus 5 μm, 150 mm × 4.6 mm i.d. (Supelco, Poole Dorset, UK) maintained at 40 °C. Elution was performed by a gradient from 100% 30 mM ammonium acetate at pH 5.5 to 50% 30 mM ammonium acetate at pH 5.5 and 50% acetonitrile in 10 min. Detection was at 440 nm. The intra-assay coefficient of variation (CV%) was between 2.2% and 7.7% and the inter-assay CV% was within 9.5%. The accuracy was within 12% at all concentrations.

Pharmacokinetic parameters were calculated by a non-compartmental model. The AUC was calculated without extrapolation to infinity by log-trapezoidal rule. Apparent bioavailability (*F*%) was calculated for the first and for the last oral dose of cycle 1 using the formula:

$$F\%_{\text{first dose}} = (\text{AUC}_{\text{norm}}\text{day } 1/\text{AUC}_{\text{norm}}\text{day} - 7) * 100 \quad \text{and}$$
$$F\%_{\text{last dose}} = (\text{AUC}_{\text{norm}}\text{day } 28/\text{AUC}_{\text{norm}}\text{day } 29) * 100$$

where AUC_{norm} is the AUC 0–12 h normalized for milligrams of administered SU006668.

The terminal half-life ($T_{1/2}$) was derived by the formula $T_{1/2} = 0.693/\text{Ke}$, where Ke was the slope of the linear equation that best fitted the last three or four concentration-time data. Total body clearance (CL_{TB}) was calculated as dose/AUC. Volume of distribution at steady state (V_{ss}) was calculated by the formula V_{ss} = dose/AUC * (AUMC/AUC) [10].

Wilcoxon's test for paired data was used to test for difference between pharmacokinetic parameters.

2.6. *SU006668 urine level measurements*

A HPLC method was developed and validated for the quantitation of SU006668 in human urine in a range of 50–25,000 ng/ml.

Urine was diluted with acetonitrile (2:1) and, after addition of SU009905 as internal standard, 50 μl were injected directly in the HPLC system. Chromatographic separation was achieved with the conditions already described for the assay of plasma samples. Chromatograms from urine samples showed several peaks with UV–Vis spectra profile similar to that of SU006668, suggesting multiple metabolic pathways.

2.7. *Protein binding*

Protein binding of SU006668 was determinated by equilibrium microdialysis using a EMD 101B-Equilibrium microvolume

dialyzer (Hoefer Pharmacia Biotech Inc., San Francisco, CA) equipped with Teflon chambers separated in two cells (0.5 ml capacity) by a dialysis membrane with 6000–8000 Da cut-off.

SU006668 (4000 ng/ml) was added to human plasma or to plasma from healthy nude mice and equilibrated for 46 h at room temperature with ultrafiltrate from human or mice plasma. Experiments were also performed equilibrating SU006668 with human serum albumin (4 g/100 ml) (Castelvecchio P., Lucca, Italy). The solutions were recovered after equilibrium and SU006668 was measured by HPLC.

3. Results

Twenty-four adult patients entered the study (Table 1). One patient was not evaluable for safety and activity because of death due to tumour progression on day 8 of the first cycle at 100 mg/m^2. Twenty-two patients had prior chemotherapy and 10 also had prior radiation. In one patient with pleural mesothelioma SU006668 was the first anticancer treatment. Eight, nine and seven patients were treated at 100, 200 and 300 mg/m^2 b.i.d, respectively. The dose of 400 mg/m^2 b.i.d. was defined as the MTD in another Phase I and escalation was discontinued. The median number of cycles was 2 (range: 1–23) with four patients receiving more than 3 cycles (23 cycles in one).

3.1. Toxicity

Two patients had possible DLT: the first patient with parotid gland adenocarcinoma developed G2 pericardial effusion at the end of the first cycle at 100 mg/m^2 b.i.d.; and a second patient with a diagnosis of hepatocarcinoma and hepatic cirrhosis with arterial and portal hypertension, developed G3 thrombocytopenia and G3 asthenia during the first cycle at 300 mg/m^2. Overall, myelotoxicity was mild and the main side-effect was that was of moderate severity in 9 patients, although only two had started therapy with normal haemoglobin values.

The most frequently reported drug-related side-effects were dose-dependent fatigue (33% of patients), diarrhoea (26%), nausea and vomiting (25%) (Table 2). No cumulative toxicity was observed and one patient with stable disease received a total of 23 cycles without worsening of side-effects.

Table 1 – Patients characteristics

Total no. of patients (median cycles; range)	24 (2; 2–23)
Patients per dose (median cycles; range)	
100 mg/m^2	8 (2; 1–4)
200 mg/m^2	9 (2; 1–23)
300 mg/m^2	7 (2: 1–7)
Age – median (range) years	55 (31–72)
ECOG performance status – median (range)	0 (0–1)
Cancer type	
Breast	5
Adenocarcinoma	6
Parotid gland	2
Colorectal	2
Other	2
Soft tissue sarcoma	9
GIST	2
Hemangioendothelioma	2
Leiomyosarcoma	2
Mesothelioma	1
pPNET	1
Unclassified	1
Osteosarcoma	1
Hepatocarcinoma	3

Abbreviations: ECOG, Eastern Cooperative Oncology Group; pPNET, peripheral primitive neuroectodermic tumor; GIST, gastro intestinal stromal tumor.

Table 2 – Non-hematological toxicities

Toxicity type	Dose and NCI CTC grade								
	100 mg/m^2			200 mg/m^2			300 mg/m^2		
	1	2	3	1	2	3	1	2	3
Anorexia	8	8						8	
Fatigue	4			8	4		13	4	
Nausea	4			17				4	
Vomiting	8			8			8		
Somnolence	4			4					
Diarrhea	4			13			8		

Percent of patients with worst NCI CTC grade by dose.
Abbreviations: NCI-CTC, National Cancer Institute Common Toxicity Criteria Version 2.0.

3.2. Antitumour activity

No objective responses were seen, but benefit was observed in a 69-year-old woman with liver metastases from a gastrointestinal stromal tumour (GIST) who had previously received anthracyclines and imatinib. The patient, who received SU006668 for 28 weeks overall, showed an increase in the necrosis of the liver metastases with decrease of the peripheral hypervascular rim (Fig. 3), associated with a subjective improvement and normalization of liver function tests lasting for eight months. Two further patients, a 56-year-old woman with an epithelioid haemangioendothelioma and a 59-year-old woman with metastatic breast cancer, had stabilization of their disease lasting, respectively, 23 and 7 months.

3.3. Pharmacokinetics

A complete characterization of the pharmacokinetics of SU006668 after oral and intravenous administration was performed in 15 patients, of whom five received an oral dose of 100 mg/m^2, four of 200 mg/m^2, and six of 300 mg/m^2. Four additional patients received 200 mg/m^2 of SU006668 PO but did not undergo intravenous testing while five more did not complete the first cycle and only samples on day −7 and 1 were collected.

The plasma concentrations of SU006668 after intravenous administration of 100 mg/m^2 could be fitted to a two-compartment model. As shown in Fig. 4, the plasma disposition was different after administration on day −7 of the drug and after four full weeks of oral dosing on day 29. In particular, the value of C_{max} immediately after the end of the infu-

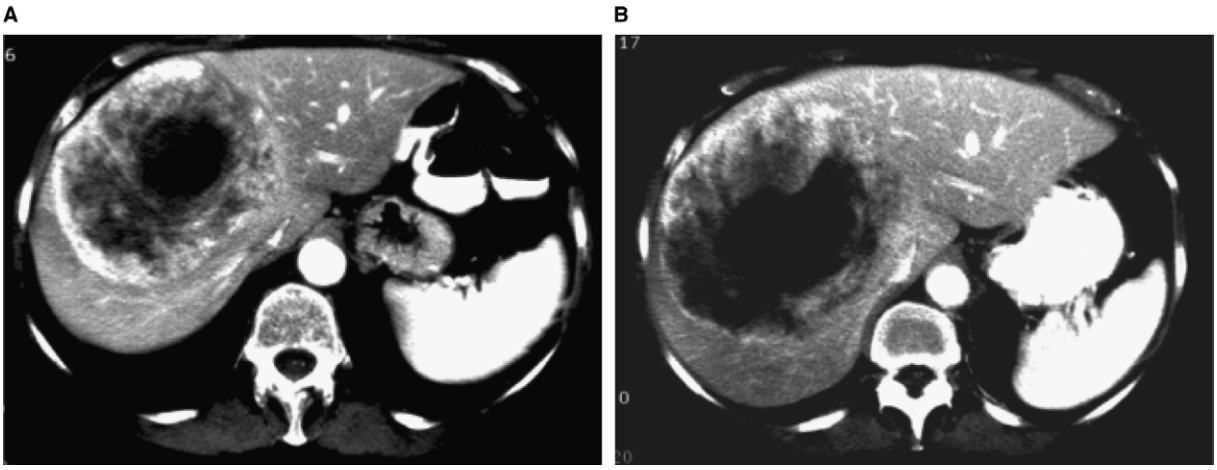

Fig. 3 – CT scan of liver metastasis from gastro-intestinal stromal tumour (GIST) in a 69-year-old woman treated with SU006668 for 28 weeks at 200 mg/m^2. (A) Baseline. (B) Post-treatment: Increased necrosis of the liver metastases with decrease of the peripheral hypervascular rim.

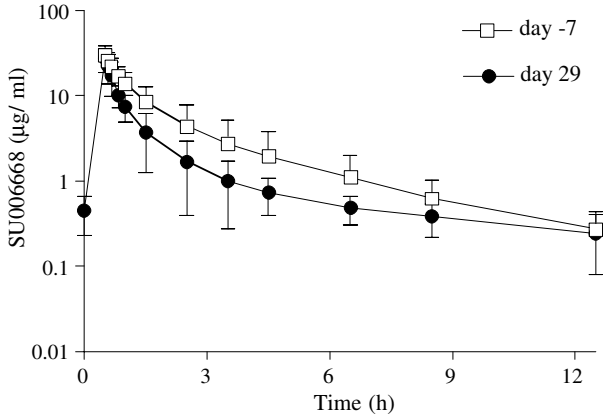

Fig. 4 – Effects of chronic oral administration on pharmacokinetic profile of intravenous SU006668 (100 mg/m^2). Empty squares represent the mean plasma concentrations measured after the intravenous dose on day −7; filled circles are relative to the intravenous dose administered on day 29 (n = 15).

Table 3 – Mean (±SD) pharmacokinetic parameters of intravenous SU006668 after 100 mg/m^2 on days −7 and 29

	Day −7	Day 29
N (number of patients)	15	15
AUC 0–24 h (µg/ml/h/m^2)	46.8 ± 19.1	30.1 ± 10.3**
C_{max} (µg/ml)	29.3 ± 8.4	26.3 ± 8.4
$T_{1/2}$ (h)	6.3 ± 2.4	10.9 ± 6.4
CLTB (L/h/m^2)	2.8 ± 1.4	4.08 ± 2.3**
V_{ss} (L)	8.8 ± 4.4	24.3 ± 16.4*

Abbreviations: SD, standard deviation; AUC, area under the plasma concentration time curve; C_{max}, maximum concentration after dose; CLTB, total body clearance; $T_{1/2}$ apparent elimination half-life; V_{ss}, steady state volume of distribution.
*$P < 0.05$.
**$P < 0.01$ by paired Wilcoxon t-test.

sion was similar, but the AUC in the first 24 h was significantly smaller on day 29, when also the clearance and the apparent volume of distribution were significantly different (Table 3). Importantly, the effect of continuous oral dosing was similar and independent of the daily oral dose administered between the two intravenous tests (data not shown).

At each dose level, the pharmacokinetic disposition after oral administration showed high inter-patient variability. Intra-patient comparison of drug disposition over time was therefore performed. Table 4 summarizes the main pharmacokinetics of SU006668 at the different tested doses when AUC was calculated in the interval between the morning and evening meal dose ($AUC_{0–12}$). At each dose, the AUC on day 28 was significantly lower than on day 1. This was apparent not only when comparing $AUC_{0–12\,h}$, but also when comparing AUC values normalized for milligrams of administered dose (Table 4). Similarly, there was a drastic reduction of the C_{max} on day 28, while trough concentrations were of the same order of magnitude. Bioavailability (expressed as apparent F%) was evaluated by comparing the ratio of the normalized AUC after oral and intravenous doses on day 1 (PO) and day −7 (iv) and on days 28 (PO) and 29 (iv). The apparent fraction absorbed F% decreased after treatment at all dose levels.

In preclinical rodent studies, the duration of exposure at concentrations larger than 2.3 µg/ml was associated with antitumour activity of the drug (4). As shown in Table 4, hours at concentrations >2.3 µg/ml were progressively increasing with higher doses on day 1 of the cycle, but no drug was seen on day 28 after 4 weeks of chronic therapy. Also the duration of the interval spent at concentrations larger than 1 µg/ml, the threshold for in vivo inhibition of Flk-1/KDR phosphorylation [2], was consistently and significantly decreasing over time at all doses of SU006668 (Table 4).

The overall urinary excretion of parent SU006668 was low. During first intravenous administration, the unmodified drug

Table 4 – Mean (±SD) pharmacokinetic parameters of SU6668 after oral administration of different doses on days 1 and 28

	100 mg/m^2		200 mg/m^2		300 mg/m^2	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
AUC 0–2 h (μg/ml·h·m^2)	16.3 ± 11.8	8.2 ± 2.9	21.6 ± 9.2	12.4 ± 3.7 *	27.6 ± 10.4	11.4 ± 3.7 **
Normalized AUC/mg of SU006668 administered (μg/ml/m^2)	0.19 ± 0.1	0.093 ± 0.04	0.11 ± 0.05	0.069 ± 0.03**	0.09 ± 0.03	0.04 ± 0.1 **
C_{max} (μg/ml)	3.17 ± 2.3	1.82 ± 0.5	4.96 ± 2.8	2.58 ± 1.3 **	5.8 ± 1.6	1.93 ± 0.8 **
C_{min} (μg/ml)	0.43 ± 0.3	0.52 ± 0.5	1.31 ± 1.1	0.97 ± 0.8	0.74 ± 0.2	0.67 ± 0.5
Apparent F (%)	42 ± 21	35 ± 17	25 ± 11	19.6 ± 9.2	26 ± 9.2	19.7 ± 8.7
Time > 2.3 μg/ml (h)	2.5	0	4	0	5.7	0
Time > 1 μg/ml (h)	6	3	7	5.2	11.0	5.8

Abbreviations: SD, standard deviation; AUC, area under the plasma concentration time curve; C_{max}, maximum concentration after dose ; C_{min}, trough concentration; *F*, bioavailability.
*$P < 0.05$.
**$P < 0.01$ by paired Wilcoxon *t*-test.

Table 5 – Urinary excretion of SU006668 and total metabolites at the start and at the end of oral chronic treatment

SU006668 (mg/m^2)	Mean (±SD) % SU006668		Mean (±SD) % total metabolites	
	Start	End	Start	End
100 IV	1.64 ± 1.4	1.00 ± 0.5*	11.9 ± 6.5	15.8 ± 11.5*
100 PO	0.61 ± 0.4	0.37 ± 0.2	4.67 ± 2.6	7.03 ± 6.7
200 PO	0.42 ± 0.4	0.29 ± 0.2	2.34 ± 1.1	4.16 ± 1.8**
300 PO	0.20 ± 0.1	0.18 ± 0.1	2.46 ± 1.3	2.37 ± 1.3

Data expressed as % of administered dose of SU006668.
Abbreviations: SD, standard deviation; IV, intravenous administration; PO, oral administration.
*$P < 0.05$.
**$P < 0.01$ by paired Wilcoxon *t*-test.

excreted in the first 12 h was 1.64 ± 1.4% of total dose (Table 5). This value decreased to 1 ± 0.5% on the iv dose of day 29, when there was a corresponding significant increase from 11.9% to 15.8% of the metabolites excreted. During oral administration the mean percent excretion of SU006668 gradually decreased with increasing dose, while excreted metabolites increased (Table 5). The urinary excretion patterns together with the plasma disposition described above are suggestive of a saturable absorption and an inducible metabolism that could explain the non-linearity of the kinetics.

3.4. *Protein binding*

The protein binding of SU006668 to human plasma was always greater than 99%, either when the drug was added to plasma and equilibrated with the same plasma's ultrafiltrate (99.5 ± 0.3%) or when added to the ultrafiltrate and equilibrated with plasma (99.4 ± 0.3%). The corresponding protein binding in mice was respectively 96 ± 0.5% and 95.8 ± 0.5%. The percentage of drug bound in presence of human serum albumin was approximately 99.3%.

4. Discussion

The results described in this report document a complex disposition of SU006668 in man, limiting the likelihood of achieving active drug concentrations, and have contributed to the discontinuation of the drug's clinical development. SU006668 is a selective inhibitor of KDR/Flk-1, of FGFR and to a lesser extent of PDGFR that was selected for clinical development because of the convenience of the oral formulation and the appealing antitumour activity reported in tumour xenografts [2,11,12]. Three Phase I trials were ongoing when the present study was designed, one with the once daily dose, one with the b.i.d. [4] and one with t.i.d [13] schedule. The main goals of the present study were to define the optimal dose of the b.i.d. schedule while extensively exploring the pharmacology of SU006668 in humans. In particular, there was interest in assessing the reasons for the apparent non-linearity of the drug's plasma disposition that emerged from the study adopting the once-daily schedule (personal communication). More importantly, the study also wanted to assess if plasma concentrations could be reached and maintained in the range corresponding to those pharmacologically active in animal models.

Based on preclinical data indicating that fasting animals had a more rapid apparent elimination than fed animals, this study started at the initial level of 100 mg/m^2 daily. This dose was already known to be well tolerated in man, and it was administered every 12 h within 1 h after food consumption. Dose escalation was discontinued at 300 mg/m^2 b.i.d. in the absence of a formal MTD because dose-limiting toxicities were observed at 400 mg/m^2 in another ongoing study. In addition, the pharmacokinetic and pharmacological characterization of the present study did not justify continuous investigation of the drug.

On clinical grounds, the results described in this report indicated that 200 mg/m^2 b.i.d. could be the recommended regimen for chronic SU006668. At this dose, main toxicities were moderate fatigue, mild anaemia, and gastro-intestinal discomfort. In addition, hints of antitumour activity were observed in a patient with a large liver mass from GIST who had not responded to prior therapy with imatinib; and eight more patients had stable disease consistent with limited benefit.

The extensive pharmacological characterization conducted in this study clarified several aspects of the disposition of SU006668. The comparison of plasma concentrations after the same intravenous dose before and after 4 weeks of daily oral SU006668 showed a decrease in the plasma exposure to the drug. Interestingly, the decrease was similar for any given dose of orally administered drug. This observation was reminiscent of what has long been known for other drugs, such as cyclophosphamide [14], and could be interpreted as indicative of induction of metabolism following chronic administration. The study of the urinary excretion suffers the limitation that SU006668 and its metabolites are mostly excreted through the bile in animals. The very small fraction of the administered dose that could be measured in the urine collection in our study suggests that in humans the kidney does not represent the main excretory organ. Nonetheless, the investigation of urinary excretion showed that the percentage of dose excreted as metabolites increased after chronic oral administration, in keeping with the hypothesis of an inducible metabolism. The plasma disposition of SU006668 during oral administration showed a very high degree of variability that prompted an intra-patient comparative analysis. The results of such analysis showed a decrease of the apparent oral availability of the drug at increasing doses as well as over time within the same dose level. In the latter case, consistently lower exposure to the drug could be measured on day 28 than on day 1 of the oral administration. A classical example of such a trend is related to the oral saturable absorption of methotrexate [15–18], and similarly could be attributed to saturation of the mechanisms responsible for absorption from the gastro-intestinal tract. Indeed, SU006668 belongs to a class of chemicals characterized by a low hepatic extraction rate, for which the clearance depends from the intrinsic enzymatic capacity of liver [10]. Therefore, the progressive decline of plasma concentration of SU006668 after chronic oral dosing also is consistent with the induction of metabolism, as already suggested by the intravenous and urinary studies, and would be in agreement with an inducible first-pass metabolic clearance in the liver.

The present study was not designed to discriminate between saturable absorption and inducible metabolism, which most likely co-exist in the disposition of SU006668 in humans. Independent of the mechanisms involved, the data indicate that increasing the dose of oral SU006668 would not cause a corresponding increase in plasma concentrations. This had relevant implications for the development of SU006668, because in murine tumour models the drug was active only if plasma concentrations were higher than 2.3 μg/ml and maintained. Our investigations showed that the time spent above that threshold progressively decreased over time and eventually was zero on day 28 after chronic dosing at all tested doses. In addition, studies on protein binding showed that SU006668 avidly bound to human plasma, most likely to albumin, for more than 99%, while binding was about 96% to plasma proteins from nude mice. It appeared that for any total plasma concentration, the free drug available for the targets of SU006668 would be 4 to 15-fold less than in mice. Other variables, such as occupancy of the target and rate of induction of avid binding to the target, could allow for building up of pharmacologic effects, and some limited benefit was observed in our study. However, the high protein binding, the complex pharmacology reported here, and the lack of relevant antitumour activity in other studies were elements in the decision to discontinue the development of SU006668 in humans and has accelerating the investigation of the analogue compound SU11248 [19–21].

A more general conclusion applicable to the development of molecular targeted treatments could also be drawn from this study. Targeted therapies are often viewed as new treatments deserving a new and different approach in early development. This study shows that the characterization of the pharmacological and pharmacokinetic profile of new targeted agents is as informative and much needed in the early phases of human testing. Such experiments will guide further development targeted drugs, as for any other putative drug, especially when the oral route is sought.

Conflict of interest statement

The authors declare that they have no financial interest which may inappropriately influence the undertaking of the study.

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