ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetics of SU5416 after intravenous administration in nonhuman primates

Abstract *Purpose*: SU5416 is a small, lipophilic synthetic molecule that selectively inhibits the tyrosine kinase activity of the VEGF receptor Flk-1/KDR. The role of this agent in brain tumors is currently being investigated. Pharmacokinetic studies of SU5416 have been performed in humans; however, there have been no studies of its penetration in the cerebrospinal fluid (CSF). We studied the pharmacokinetics of SU5416 in plasma and CSF after intravenous (i.v.) administration using a nonhuman primate model that is highly predictive of the CSF penetration in humans. *Experimental design*: SU5416 (85 mg/m^2, about 3.8 mg/kg) was administered i.v. over 20 min to four nonhuman primates. Serial plasma and CSF samples were obtained prior to, during, and after completion of the infusion for determination of SU5416 concentrations. SU5416 was measured in plasma and CSF using high-performance liquid chromatography (HPLC). Concentration-versus-time data were modeled using model-independent and model-dependent methods. *Results*: Peak plasma concentrations ranged from 6.3 to 14.5 μM and the mean plasma AUC was 620 ± 180 μM·min. Disappearance of SU5416 from the plasma was best described by a one-compartment model with a half-life of 39 ± 2.9 min. The volume of distribution was 36 ± 11 l/m^2 and the clearance was 0.62 ± 0.2 l/min per m^2. SU4516 was not quantifiable in the CSF. *Conclusions*: There is minimal penetration of SU5416 into the CSF after i.v. administration. The very low CNS exposure to SU5416 after i.v. dosing suggests that this agent is not optimal for the treatment of leptomeningeal tumors.

Keywords SU5416 · Antiangiogenesis Leptomeningeal malignancy

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Introduction

SU5416 (Z-3-[(2,4-dimethylpyrrol-5-yl)methylidenyl]-indoline-2-one; Fig. 1) is a small, lipophilic synthetic molecule that selectively inhibits the tyrosine kinase activity of the VEGF receptor Flk-1/KDR [4, 8]. In preclinical studies, SU5416 has been shown to have no effect on tumor cells in vitro but causes dose-dependent inhibition of tumor growth in vivo [7]. SU5416 has shown activity in many different tumor xenografts in nude mice including melanoma, glioma, fibrosarcoma and lung, and in epidermoid, mammary, and prostate carcinomas [7]. In addition, Shaheen et al. studied the effect of SU5416 on tumor angiogenesis and metastasis in a human colon cancer xenograft model [12]. They demonstrated that SU5416 inhibits tumor metastases, microvessel formation, and cell proliferation. The findings show that SU5416 inhibition of the VEGF receptor system decreases tumor vascularity and vessel density and increases tumor cell apoptosis. The results indicate that targeting VEGF receptor is a rational approach to inhibiting tumor growth and prolonging survival [3].

Initial phase I and II clinical studies of SU5416 have been completed [1, 11] and phase III trials are in progress. Although the plasma pharmacokinetics of SU5416 have been well characterized, there are no detailed studies of the cerebrospinal fluid (CSF) disposition of SU5416. Therefore, we evaluated the plasma and CSF pharmacokinetics of SU5416 in a nonhuman primate model that is highly predictive of the CSF penetration of anticancer drugs in humans [6].

Materials and methods

Drug

SU5416 was supplied by SUGEN (South San Francisco, Calif.) in 25-ml vials at a concentration of 4.5 mg/ml in Cremophor EL, which includes polyethylene glycol 400/Polyoxyl 35 castor oil in addition to benzyl alcohol and dehydrated alcohol. Vials were stored at room temperature and protected from the light. The drug
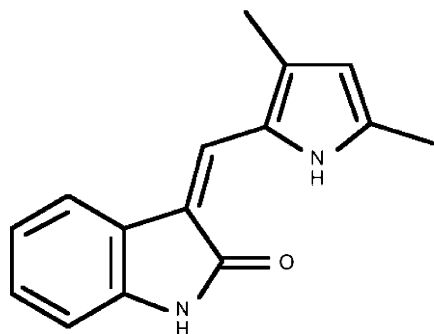
Fig. 1 Chemical structure of SU5416

One compartment model

Fig. 2 One-compartment pharmacokinetic model

was further diluted to a final concentration of 1.5 mg/ml with normal saline and administered through non-PVC tubing. The bag was gently inverted to ensure complete mixing of the solution. Animals were premedicated with dexamethasone at a dose of 1 mg/kg. The drug was then administered at a rate of 100 ml/h.

Animals

Five total doses of SU5416 were administered to four adult male rhesus monkeys (*Macaca mulatta*), ranging in weight from 12.3 to 13.6 kg, in these pharmacokinetic studies. The animals were fed NIH Open Formula Extruded Non-human Primate Diet twice daily and were group housed in accordance with the Guide for Care and Use of Laboratory Animals [9]. Blood samples were drawn through a catheter placed in either the femoral or saphenous vein contralateral to the site of drug administration. CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a subcutaneously implanted Ommaya reservoir. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

Four animals received a 3.8 mg/kg (about 85 mg/m^2) dose of SU5416 administered via a central venous access device at a rate of 100 ml/h. The infusion durations ranged from 19 to 20.7 min. Blood was collected in heparinized tubes prior to the dose, at 10 min during the infusion, at the completion of the infusion, and at approximately 5, 15 and 30 min, and 1, 2, 3, 4, 5, 7, 8 and 24 h following completion of the infusion. Plasma was separated immediately by centrifugation at 3310 *g* for 10 min. The CSF samples were collected prior to the dose, at 10 min during the infusion, at the completion of the infusion, and at 15 and 30 min, and 1, 2, 3, 4, 5, 7 and 8 h following completion of the infusion. The plasma and CSF samples were stored at −80°C until the day of analysis.

Sample processing and analysis

SU5416 was measured using reverse-phase high performance liquid chromatography (HPLC). Plasma samples were thawed and then centrifuged for 10 min at 16,000 *g* prior to the solid-phase extraction. A Varian Bond-Elut 100 mg C8 solid-phase extraction column was conditioned with 1 ml methanol followed by 1 ml water. A 1-ml aliquot of the plasma was then placed on the cartridge. The cartridge was washed with 1 ml water followed by 1 ml 10% acetonitrile. The SU5416 was eluted with 1 ml 100% acetonitrile. The samples were then dried under a gentle flow of nitrogen. The residues were reconstituted in 500 μl of a solution of acetonitrile/DMSO/10 m*M* ammonium acetate (pH 2.6, ratio 4:2:3). A 25-μl aliquot was injected onto the HPLC system as described below.
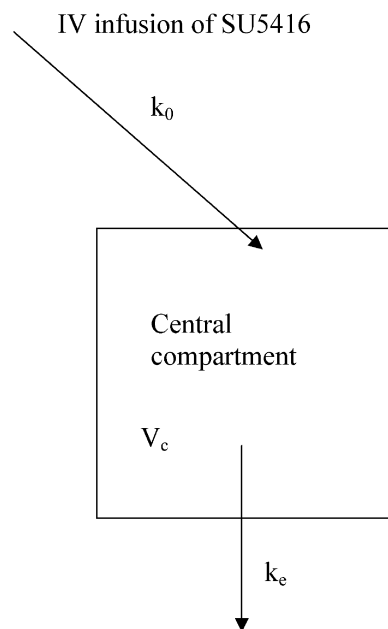
The recovery of SU5416 was 99.6 ± 0.7%. Standard curves for both plasma and CSF were constructed by the addition of known amounts of SU5416 to plasma or phosphate-buffered saline, respectively. The CSF samples were thawed and centrifuged at 16,000 *g* for 10 min followed by direct injection of 50 μl onto the system without further processing. Standard curves were linear ($r^2 > 0.995$) over the range 0.04 to 210 μ*M* for the plasma curve and 0.02 to 4.2 μ*M* for the CSF curve. The lower limit of quantitation was 0.04 μ*M* for plasma and 0.02 μ*M* for CSF.

The HPLC system consisted of a Waters 600 Controller, a Waters 490E programmable multiwavelength UV detector with a reverse-phase NovaPak 4 μm C8 (3.9×150 mm) analytical column. SU5416 was eluted isocratically with a mobile phase of 50% acetonitrile/50% 10 m*M* ammonium acetate (pH 2.6) at a flow rate of 1 ml/min. SU5416 was monitored at a wavelength of 440 nm and had a retention time of 6.2 min.

Pharmacokinetic analysis

One- and two-compartment models were fitted to the plasma concentration-versus-time data from the individual SU5416 i.v. infusion experiments using Adapt II [13] (Fig. 2). Akaike's information criterion were used to determine which equation best fitted the data. Other pharmacokinetic parameters (clearance, volume of distribution at steady state, and half-lives) were derived from the estimates of model parameters using standard techniques [13]. The areas under the concentration-time curve (AUC) were determined by the linear trapezoidal method [13] and extrapolated to infinity using the terminal rate constant.

Evaluation for toxicity

Clinical laboratory studies including complete blood counts, electrolytes, liver function tests, and renal function tests were obtained on a weekly basis for a minimum of 3 weeks each SU5416 infusion. Animals were also observed on a daily basis for a minimum of 3 weeks after infusion for any evidence of clinical toxicity.

Fig. 3 Representative plasma concentration-time profile of SU5416 following a 20-min i.v. infusion to nonhuman primates
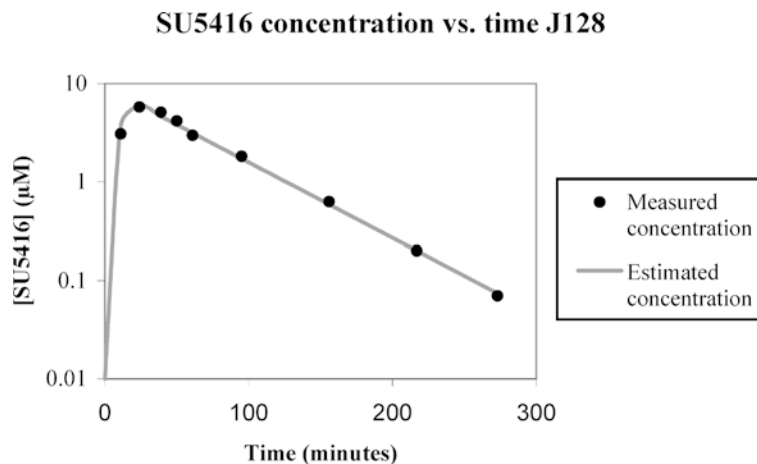

SU5416 concentration vs. time J128

Table 1 Plasma pharmacokinetic parameters of SU5416 after i.v. infusion of 3.8 mg/kg over 20 min in nonhuman primates

Animal	AUC_p ($\mu M\cdot$min)	V_c (l/m^2)	K_e (min^{-1})	CL (l/min/m^2)	$t_{1/2}$ (min)
L984	435	45	0.019	0.83	37
J128	441	48	0.018	0.85	39
J0A	651	38	0.014	0.53	38
J0B	835	26	0.016	0.42	44
L962	752	24	0.020	0.48	35
Mean ± SD[a]	620 ± 180	36 ± 11	0.017 ± 0.002	0.62 ± 0.21	39 ± 2.9

[a] $t_{1/2}$ (min) expressed as harmonic mean ± SE

Results

Pharmacokinetics

The elimination of SU5416 from plasma was best described by a one-compartment model. A representative plasma concentration-time profile is shown in Fig. 3. The concentration of SU5416 in the CSF was not quantifiable. The pharmacokinetic parameters for the i.v. infusion are listed in Table 1. AUC, V_c, K_e, and CL are expressed as the mean values ± SD; $t_{1/2}$ is expressed as the harmonic mean.

The AUC of SU5416 in the plasma was 620 ± 180 $\mu M\cdot$min. SU5416 was rapidly eliminated from the plasma with a $t_{1/2}$ of 39 ± 2.9 min. The CL_{TB} of SU5416 was 0.62 ± 0.21 l/min per m^2 and the volume of distribution was 36 ± 11 l/m^2.

Toxicity

SU5416 was well tolerated. There were no significant hematologic or other organ toxicities after a single i.v. infusion of drug. One animal developed mild, self-limited lethargy following administration of the drug. No other acute or chronic toxicity was observed.

Discussion

We studied the plasma and CSF kinetics of SU5416 in a nonhuman primate model that has been highly predictive of CSF drug penetration in humans [10]. Elimination of SU5416 from plasma was best described using a one-compartment model with a half-life of 39 ± 2.9 min and a clearance of 0.62 ± 0.21 l/min per m^2. These plasma pharmacokinetic parameters are similar to previously reported preclinical and clinical results [1, 5, 11, 14]. Studies of SU5416 pharmacokinetics in humans at doses of 4.4 to 190 mg/m^2 at the same infusion rate utilized in this study have shown an elimination half-life of approximately 50 min, a volume of distribution of approximately 22 l/m^2, and a clearance of approximately 52 l/h (0.87 l/min) [11]. Pharmacokinetic studies of SU5416 reported in the literature have shown a two-compartment model for this drug [1, 5, 11].

In this study, SU5416 was not quantifiable in the CSF. SU5416 is more than 99% protein-bound (SUGEN Investigator Brochure). It is well established that protein-bound drug does not readily cross cell membranes and consequently has a restricted distribution [13]. Furthermore, because the protein content of the CSF is low relative to the amount of protein in the blood, CSF equilibrates with the free fraction of drug across a relatively impermeable membrane. Therefore minimal penetration of SU5416 into the CSF was not an unexpected finding.

SU5416 is currently being studied in clinical trials for the treatment of a variety of different types of malignancies including brain tumors. The ability of drugs to penetrate into the CSF has been used as a surrogate for penetration across the blood-brain barrier into the central nervous system. However, many brain tumors have a disrupted blood-brain barrier as evidenced by the fact that these tumors take up gadolinium, which does not cross the intact blood-brain barrier [2]. Thus the limited penetration of SU5416 into the CSF does not preclude antitumor activity against CNS tumors. Given the fact that there was no measurable penetration of SU5416

into the CSF following i.v. infusion, however, this agent may not be optimal for the treatment of leptomeningeal tumors.

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