

## SHORT COMMUNICATION

## THE PENETRATION OF SUNITINIB THROUGH THE BLOOD-BRAIN BARRIER AFTER THE ADMINISTRATION OF CIPROFLOXACIN

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and EDMUND GRZEŚKOWIAK<sup>1</sup><sup>1</sup>Department of Clinical Pharmacy and Biopharmacy, <sup>2</sup>Department of Teaching Anaesthesiology and Intensive Therapy, Poznan University of Medical Sciences, Św. Marii Magdaleny 14, 61-861 Poznań, Poland<sup>3</sup> Polpharma Biologics, Trzy lipy 3, 80-172 Gdańsk, Poland**Keywords:** sunitinib, blood-brain barrier, ciprofloxacin, P-gp, rabbits

Sunitinib (SU 11248) is an efficacious antineoplastic and antiangiogenic drug from the group of tyrosine kinase inhibitors (TKIs). In 2006, SUTENT<sup>®</sup> (Pfizer) was registered by the US Food and Drug Administration (FDA) for the treatment of gastrointestinal stromal tumor (GIST) if the imatinib therapy is unsuccessful and for the treatment of renal cell carcinoma (RCC) (1). In 2011, SUTENT<sup>®</sup> was recommended by the FDA for the treatment of progressive pancreatic neuroendocrine tumors (2). Apart from the indications listed above, the drug may also be efficacious in non-small-cell lung cancer, breast cancer and neuroendocrine neoplasms (3). The drug achieves its antineoplastic effect by inhibition of the following receptors: platelet-derived growth factor receptors (PDGFR $\alpha$  and PDGFR $\beta$ ), vascular endothelial growth factor receptors (VEGFR1, VEGFR2, VEGFR3), stem cell factor receptor (KIT), Fms-like tyrosine kinase receptor-3 (FLT3), colony stimulating factor receptor (CSF-1R), glial cell line-derived neurotrophic factor receptor (RET). The pharmacokinetics of sunitinib was studied in a number of investigations. The bioavailability of the drug in animals is about 50% (4). The maximum concentration of the drug in the plasma ( $C_{max}$ ) is observed between 6 and 12 h after the administration (5). A daily dose of 50 mg of sunitinib gives a possibility to reach the steady-state

concentration of 50–100 ng/mL, which influences the pharmacological effect of the drug (6). The time necessary to achieve the steady state is 7–14 days for sunitinib and about 14 days for its active metabolite SU 12662 (N-desethylsunitinib). The administration of the drug once a day for 14 days causes the concentration of sunitinib to increase 4.5 times; the concentration of the active metabolite increases 10 times and the complete fraction of the drug (sunitinib plus SU 12662) increases 5 times (7). CYP3A4 has been proved to be involved in the metabolism of sunitinib and its major circulating metabolite (SU 12662) (5, 6, 8), which unfortunately may be the cause of interaction with the inhibitors or inducers of this enzyme when they are simultaneously applied (9).

At present, there are only a few publications available, which support the opinion that sunitinib may cross the blood-brain barrier. However, few authors confirm sunitinib activity in brain metastases from kidney cancer (10–13). Patyna et al. (14) showed rapid distribution of [<sup>14</sup>C]-sunitinib and its metabolite in the brain and spinal cord tissue after intravenous or oral administration of the drug to monkeys, rats and mice. The conclusion of the research was the need to continue it due to the anti-tumor activity in the brain. There is growing interest in the subject because of the efficaciousness of sunitinib

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tinib in the group of patients with brain metastases from kidney cancer. P-glycoprotein (P-gp; ABCB1) plays a significant role in the transport of drugs through the blood-brain barrier (15–17). Numerous data demonstrated that P-gp inhibition mediated by some drugs could significantly increase brain fluid concentrations by increasing its brain permeability (18, 19). Sunitinib and SU12662 are the substrates of ABCB1 (20, 21). Increased permeability of sunitinib to the central nervous system (CNS) was observed in mice void of ABCB1 protein (20). Tang et al. proved higher accumulation of sunitinib in the brain when it was administered with elacridar, P-gp inhibitor. However, this effect was not proved for SU12662 (22).

Ciprofloxacin is used in the treatment of infections caused especially by most Gram-negative pathogens. It is still highly effective against *Pseudomonas aeruginosa*. The antibiotic is less active against Gram-positive pathogens (23, 24). Because of its good tissue penetration the fluoroquinolone is efficacious in the treatment of urinary tract infections, skin and bone infections, gastrointestinal infections, lower respiratory tract infections, febrile neutropenia, intraabdominal infections (23, 25, 26). Ciprofloxacin is an inhibitor of cytochrome P450 CYP3A4 and causes numerous drug interactions (27–30). The authors proved a significant increase in the area under the curve (AUC) and  $C_{max}$  of sunitinib after the administration of the TKI with ciprofloxacin to rabbits (31). The literature does not provide unequivocal data concerning the influence of ciprofloxacin on P-gp (32). Such fluoroquinolones as grepafloxacin, levofloxacin and sparfloxacin inhibited the efflux of erythromycin *in vitro* (33). deLange et al. proved the inhibition of ciprofloxacin on the transport of rhodamine, a P-gp substrate (34).

The current study was conducted to investigate the inhibitory effect of ciprofloxacin, a widely used fluoroquinolone, on the penetration of sunitinib into the cerebrospinal fluid. It is thought that the inhibition of P-gp at the blood-brain barrier may increase the cerebrospinal penetration of sunitinib and in consequence increase its effectiveness in the treatment of brain metastases from kidney cancer. Due to the fact that there is high probability of combination of both drugs in patients with renal cancer, ciprofloxacin was used in the research.

We searched the bibliographic database of the National Library of Medicine (MEDLINE®) and found no evidence of the effect in the literature.

## EXPERIMENTAL

### Reagents

Sunitinib and SU12662 were purchased from LGC Standards (Łomianki, Poland), HPLC grade acetonitrile, ammonium acetate and acetic acid from Sigma-Aldrich and methanol from Merck. Water used in the mobile phase was deionized, distilled and filtered through a Millipore system before use. SUTENT® was purchased (batch number P177H) from Pfizer Trading Polska Sp. z o.o., Warszawa, Poland.

### Animals

Adult New Zealand male rabbits, weighing 2.5–3.4 kg, were used for experiments. All the rabbits were kept in individual metal cages located in the animal laboratory of the Poznań University of Medical Sciences, Department and Unit of Clinical Pharmacy and Biopharmacy. They were acclimatized for two weeks prior to the experiments and were maintained under standard conditions of temperature ( $23 \pm 2^\circ\text{C}$ ) and humidity (56–60%) with an alternating 12 h light/dark cycles. New Zealand rabbits were provided with 100 g of commercial pelleted diet (Labofeed KB®: 9.8 MJ/kg metabolic energy, 16.00% total protein, 0.65% vitamin P, 15,000 IU vitamin A, 1500 IU vitamin D<sub>3</sub>, and 65 mg vitamin E) and tap water *ad libitum*. All experimental procedures related to this study were approved by the Local Ethics Committee of Poznań University of Medical Sciences.

### Evaluation of sunitinib and SU12662 pharmacokinetics

The rabbits were divided into two groups (7 animals in each): I (control) – receiving sunitinib, II – receiving sunitinib and ciprofloxacin. Ciprofloxacin (Proxacin® 10 mg/mL; Polfa, Warszawa, Poland) as solution was administered over 30 min intravenously with an infusion pump through the left marginal ear vein at the doses of 20 mg/kg/12 h (35). The steady-state was achieved by multiple administration of ciprofloxacin. After administration of the 7<sup>th</sup> dose of the antibiotic, sunitinib (SUTENT® 25 mg) was administered *per os* (*p.o.*) at the single dose of 25 mg (36). The serum and CSF samples were taken from one rabbit at the particular time points. The rabbits were anesthetized with intramuscular ketamine (30 mg/kg) and xylazine (4 mg/kg) into thigh. Ten minutes later, after the onset of anesthesia, the animals were placed in a lateral recumbent position in order to maintain the patent airways. The rabbits' respiratory

movements were continuously observed to detect the symptoms of respiratory insufficiency. During the anesthesia, an 18-G catheter was inserted into the central auricular artery for blood sampling. The hair above the neck skin was removed and the skin surface was disinfected. In the lateral recumbent position, the head was flexed and the landmarks for needle placement (the occipital protuberance and the cranial margins of the wings of the atlas) were palpated. A 20G (0.9 × 40 mm) sterile needle (Terumo Europe N.V.) needle was inserted gently in the mid-line at 90 degrees to the vertebral column, and 0.5 mL of the cerebrospinal fluid (CSF) was collected into a plastic container (SafeSeal microtube, Sarstedt). No syringe aspiration was applied. After CSF sampling, the animals were euthanized with intravenous sodium pentobarbital (Morbital, Vetoquinol-Biowet) 1 mL/kg administered into the marginal ear vein.

Blood (3 mL) and CSF (100 µL) samples for sunitinib, SU12662 assays were collected before and 2, 4, 6, 8, 12, 24 h following sunitinib administration. The blood samples were transferred into heparinized tubes and they were centrifuged at 4000 rpm for 8 min at 4°C. Next, the plasma was transferred to propylene tubes and stored at -20°C until analysis.

The measurement of sunitinib concentration in the blood plasma and CSF was made by means of the HPLC (high-performance liquid chromatography) method with UV detection, which was a modi-

fication of the method developed by Faivre et al. (37). Separation was achieved by isocratic elution of the mobile phase, ammonium acetate 20 mM pH 3.4 (adjusted with acetic acid) – acetonitrile (60 : 40, v/v), at a flow rate of 1.0 mL/min through a Symmetry® RP-C8 column (250 mm × 4.6 mm, 5.0 mm particle size) (Waters®). The column temperature was maintained at 40°C, the UV detection wavelength was set at 431 nm. The total analysis time for each run was 6 min. The lower limit of quantification (LLOQ) and limit of detection (LOD) for sunitinib and SU12662 were 1.0 ng/mL and 0.5 ng/mL, respectively. Intra- and inter-day precision and accuracy of the low quality control (2.5 ng/mL), medium quality control (25.0, 125.0 ng/mL), and high quality control (45.0, 200.0 ng/mL) were well within the acceptable limit of 10% coefficient of variation (CV%) for SU12662 and sunitinib, respectively. The calibration for sunitinib was linear in the range 1.0–250.0 ng/mL ( $r = 0.999$ ), for SU12662 in the range 1.0–50.0 ng/mL ( $r = 0.998$ ).

#### Pharmacokinetics analysis

Pharmacokinetic parameters for sunitinib and its metabolite were estimated by non-compartmental methods using validated software (Phoenix™ WinNonlin® 6.3; Certara L.P., USA). The following pharmacokinetic parameters were calculated for sunitinib in the blood and cerebrospinal fluid: maximum observed plasma concentration ( $C_{max}$ ); time to reach maximum concentration ( $t_{max}$ ); area under the

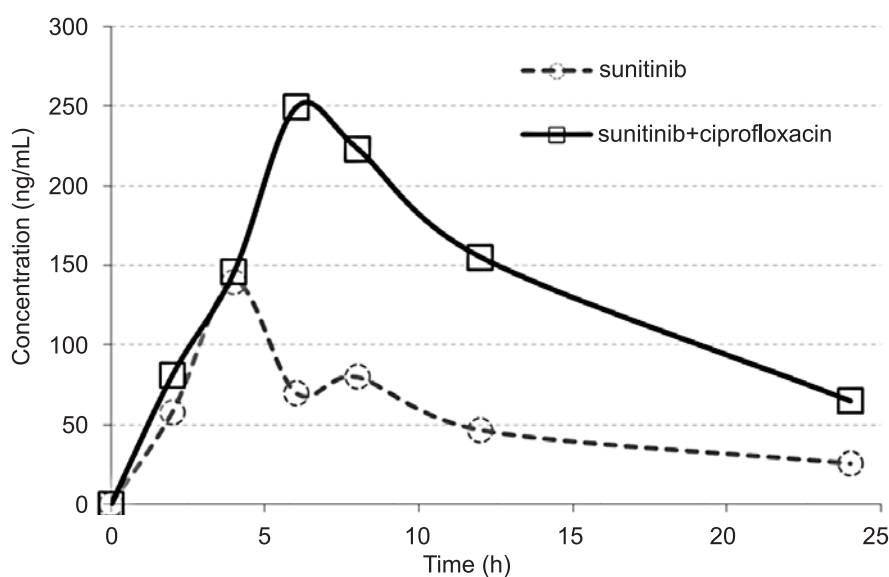


Figure 1. Sunitinib plasma concentration–time profiles following a single oral dose of sunitinib 25 mg in rabbits

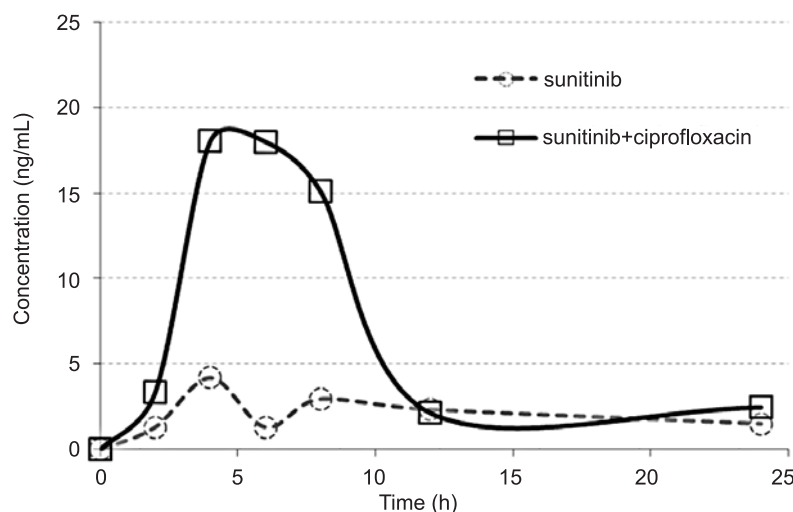


Figure 2. Sunitinib cerebrospinal concentration–time profiles following a single oral dose of sunitinib 25 mg in rabbits

plasma concentration–time curve from zero to 12 h ( $AUC_{0-12\text{ h}}$ ); area under the plasma concentration–time curve from zero to 24 h ( $AUC_{0-24\text{ h}}$ ), area under the plasma concentration–time curve from time zero to infinity ( $AUC_{0-\infty}$ ), half-life in elimination phase ( $t_{1/2\text{el}}$ ), clearance (CL), area under the first moment curve ( $AUMC_{0-24\text{ h}}$ ), mean residence time (MRT).

## RESULTS

The two analyzed groups under study did not differ significantly in body mass.

Plasma  $C_{\text{max}}$  for sunitinib in the control and sunitinib + ciprofloxacin groups were 139.5 and 248.9 ng/mL, respectively (Table 1). Cerebrospinal  $C_{\text{max}}$  for sunitinib in the control and sunitinib + ciprofloxacin groups were 4.2 and 18.0 ng/mL, respectively. Plasma  $AUC_{0-24\text{ h}}$  for sunitinib in the control and sunitinib + ciprofloxacin groups were 1304.6 and 3254.2 ng  $\times$  h/mL, respectively. Cerebrospinal  $AUC_{0-24\text{ h}}$  for sunitinib in the control and sunitinib + ciprofloxacin groups were 50.4 and 155.9 ng  $\times$  h/mL, respectively (Table 1). All calculated pharmacokinetic parameters for both groups are presented in Table 1. There was no measurable levels of metabolite in CSF.

The coefficient of drug penetration through the blood–brain barrier was estimated from the ratio of the sunitinib concentration in CSF over the plasma concentration ( $C_{\text{CSF}}/C_{\text{plasma}}$ ). The coefficients of sunitinib penetration through the blood–brain barrier are not quite similar (range 0.019–0.059 vs. 0.014–0.123 for group I and II, respectively).

The concentrations of sunitinib in the blood plasma for the two groups are shown in Figure 1. The concentrations of sunitinib in the cerebrospinal fluid in analyzed rabbits are shown in Figure 2.

## DISCUSSION

The blood–brain barrier is developed to maintain and regulate the microenvironment of the CNS and is composed of the endothelial tight junctions, which are distant to the epithelial ones. The molecular components include the set of proteins: claudins, occludin, ZO-1, 2, 3, cingulin and 7H6 creating the barrier and being responsible for the communication with the external environment using G-proteins, serine-, threonine- and tyrosine-kinases, calcium levels, cAMP, proteases and cytokines (38). Astrocytes contact the subendothelial basal lamina creating orthogonal arrays of particles (OAPs) containing the water channel protein aquaporin-4 mediating the water movement between the compartments. The blood–brain barrier is extensively disturbed in area of the brain tumors (e.g., glioblastomas, astrocytomas) thus allowing some drugs, normally not able to reach the main bulk of gliomas, to cross the barrier. P-gp is an important component of the blood–brain barrier. Blood capillaries are rich in ABCB1, and the protein takes part in the transport of numerous substances. It exhibits affinity for a broad spectrum of lipophilic substances, which are not structurally related with each other (e.g., digoxin, cyclosporin A, HIV protease inhibitors) (39–41). On the one hand, its effect is reduced exposure of

Table 1. Plasma and CSF pharmacokinetic parameters for sunitinib and metabolite (SU12662) following a single oral dose of sunitinib 25 mg.

Pharmacokinetics parameters <sup>a</sup>	I			II		
	CSF	plasma	CSF/plasma ratio	CSF	plasma	CSF/plasma ratio
Sunitinib						
AUC <sub>0-12h</sub> (ng×h/mL)	27.2	869.2	0.031	128.2	1932.6	0.066
AUC <sub>0-24h</sub> (ng×h/mL)	50.4	1304.6	0.039	155.9	3254.2	0.048
AUC <sub>0-∞</sub> (ng×h/mL)	88.0	1698.0	0.052	185.4	4111.1	0.045
C <sub>max</sub> (ng/mL)	4.2	139.5	0.030	18.0	248.9	0.072
t <sub>max</sub> (h)	4.0	4.0	1.0	4.0	6.0	0.667
MRT (h)	11.3	9.4	1.20	8.3	10.5	0.762
AUMC <sub>0-24h</sub> (ng×h <sup>2</sup> /mL)	570.1	12317.4	0.046	1297.0	34106.7	0.038
SU12662						
AUC <sub>0-24h</sub> (ng×h/mL)	-	212.3	-	-	258.2	-
AUC <sub>0-∞</sub> (ng×h/mL)	-	274.6	-	-	311.1	-
C <sub>max</sub> (ng/mL)	-	12.4	-	-	33.3	-
t <sub>max</sub> (h)	-	8	-	-	8	-
AUMC <sub>0-24h</sub> (ng×h <sup>2</sup> /mL)	-	2362.7	-	-	2611.4	-

I, sunitinib; II, sunitinib + ciprofloxacin; AUC<sub>0-12h</sub> – area under the plasma concentration-time curve from zero to 12h; AUC<sub>0-24h</sub> – area under the plasma concentration-time curve from zero to 24h; AUC<sub>0-∞</sub> – area under the plasma concentration-time curve from zero to infinity; C<sub>max</sub> – maximum observed plasma concentration; t<sub>max</sub> – time to reach maximum concentration; MRT – mean residence time; AUMC<sub>0-24h</sub> – area under the first moment curve; CSF, cerebrospinal fluid

the brain to toxic compounds. However, on the other hand, the activity of P-gp may lead to lower effectiveness of the treatment of brain tumors, for example. Thus, it seems logical that the application of a combined therapy with a P-gp inhibitor should result in better availability of the antineoplastic drug in the brain and improved clinical efficacy in diseases of the CNS. The aim of the research was to determine the influence of ciprofloxacin on the penetration of sunitinib into the CSF. The influence of ciprofloxacin on P-gp has not been clearly determined. Park et al. explain that the application of different cell lines with P-gp overexpression may lead to different, usually contradictory, conclusions whether or not a specific drug is a substrate of P-gp. In his research Park concludes that some cell lines (MDCKI and MDCKI-MDR1) indicate that ciprofloxacin is a substrate of P-gp, but data from other cells (MDCKII, MDCKII-MDR1, LLC-PK1 and L-MDR1) contradict it. Brain accumulation of sunitinib and its metabolite is restricted by ABCB1 (23). Additionally, sunitinib inhibits the function of the ATP-Binding Cassette (ABC) transporter P-gp (42), which may have influence on the availability of simultaneously applied drugs (42).

In the group of animals with ciprofloxacin the C<sub>max</sub> of sunitinib in the CSF was 329% higher than

in the control group, whereas AUC<sub>0-24h</sub> was 209% higher, which resulted from the higher parameters of C<sub>max</sub> and AUC of sunitinib in the blood in the group with the antibiotic (Table 1). Higher plasma concentrations of active metabolite SU12662 correspond with higher plasma levels of sunitinib. There was no measurable levels of metabolite in CSF. The obtained values of the pharmacokinetic parameters in the plasma for sunitinib confirm the results obtained by the authors in their earlier research on the influence of fluoroquinolones (ciprofloxacin, levofloxacin, moxifloxacin) on the pharmacokinetics of sunitinib (31), where values of mean maximum TKI concentration in the sunitinib + ciprofloxacin group and in the control group were 203.8 ± 50.6 vs. 111.8 ± 20.9 ng/mL, respectively. The results point to the inhibiting effect of ciprofloxacin on CYP3A4.

In view of the fact that in both analyzed groups the coefficients of sunitinib penetration through the blood-brain barrier are very low we can suppose that the influence of ciprofloxacin on P-gp was not significant.

The research was limited by a small number of animals. Nevertheless, even with this number of animals we succeeded in proving visible differences in the PK parameters between the groups. The higher con-

centration of sunitinib in the CSF is the consequence of higher TKI concentration in the plasma, which probably results from the inhibiting effect of ciprofloxacin on the metabolism of sunitinib. The values of the coefficients of TKI penetration through the blood-brain barrier in both groups seem to indicate that the antibiotic has little influence on P-glycoprotein.

## CONCLUSION

The study revealed increased concentrations of sunitinib in the cerebrospinal fluid after the administration of ciprofloxacin, but probably it was an effect of the inhibition of CYP3A4 by the antibiotic and increased sunitinib concentrations in the plasma rather than its influence on P-gp.

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