European Review for Medical and Pharmacological Sciences 2016; 20: 166-173

The influence of the time-of-day administration of sunitinib on the penetration through the blood-brain and blood-aqueous humour barriers in rabbits

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Abstract. – OBJECTIVE: Sunitinib is a multiple tyrosine kinase inhibitor (TKI) that exerts anti-tumor and antiangiogenic activity. It is used for the treatment of metastatic gastrointestinal stromal tumours, renal cell carcinoma and pancreatic neuroendocrine tumours. A few studies confirm the anti-tumour activity of sunitinib in brain tumours and uveal melanoma, as well as its efficacy in the reduction of brain metastases of some primary cancers. Therefore, the penetration of sunitinib through the blood-brain barrier (BBB) and blood-aqueous humour barrier (BAB) is an issue of growing interest. The aim of the study was to investigate the influence of the time-of-day administration on the penetration of sunitinib into the cerebrospinal fluid (CSF) and aqueous humour (AH).

MATERIALS AND METHODS: The rabbits were divided into two groups: I (control group) – receiving sunitinib at 8 a.m., and II – receiving sunitinib at 8 p.m. Sunitinib was administered p.o. at a single dose of 25 mg. The concentrations of sunitinib and its active metabolite (SU12662) in the plasma, CSF, AH were measured with the validated HPLC-UV method.

RESULTS: The plasma AUC_{0-t} for sunitnib in group I was 2051.8 ng × h/mL, whereas in group II it was 3069.3 ng × h/mL. The aqueous humour AUC_{0-t} for sunitinib in the groups were 43.2 and 76.3 ng × h/mL, respectively. The cerebrospinal AUC_{0-t} for sunitinib in groups I and II were 55.5 and 66.3 ng × h/mL, respectively.

CONCLUSIONS: After the evening administration (8 p.m.) the exposure to sunitinib in the rabbits' plasma, AH and CSF was higher than after the morning administration (8 a.m.), but the degree of sunitinib penetration through the BAB and BBB was very low (< 5%) and comparable in both groups.

Key Words:
Sunitinib, Blood-brain barrier, Blood-aqueous humour barrier, Rabbits.

Introduction

Sunitinib is a small-molecule tyrosine kinase inhibitor (TKI) used for the treatment of metastatic gastrointestinal stromal tumours, renal cell carcinoma and pancreatic neuroendocrine tumours in adults. It inhibits multiple receptor tyrosine kinases, which mediate cellular signalling pathways involved in the growth of cancer cells, neoangiogenesis, tumour progression and metastases. Sunitinib effectively blocks vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs), stem cell factor receptor (c-KIT), Fms-like tyrosine kinase-3 (FLT3), colony stimulating factor receptor (CSF-1R) and the glial cell-line derived neurotrophic factor receptor (RET)[1]. Sunitinib is metabolized primarily to its N-desethyl metabolite (SU12662), which has inhibitory activity similar to the parent drug[2]. The broad spectrum of targeted kinases results in the potency against many cancers. A few studies

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demonstrated the anti-tumour activity of sunitinib in the treatment of brain tumours[3-5] and uveal melanoma[6,7].

The blood-brain barrier (BBB) is a major interface between the circulating blood and central nervous system (CNS), which selectively restricts the penetration of many substances into the brain. The presence of the BBB is the main reason why many drugs exhibit no therapeutic activity in the brain. Increasing the transport of anticancer drugs through the blood-brain barrier gives the possibility to improve the efficacy of treating cancer metastases into the brain, where classic chemotherapy is of limited significance and surgical treatment is not always possible. In many cases the use of TKIs enables rapid improvement and the reduction of neurological symptoms. So far there have been many examples proving the promising efficacy of sunitinib in the reduction of brain metastases from renal cell carcinoma[8-10]. However, the ability of sunitinib to cross the blood-brain barrier is limited. Recent studies revealed that its penetration to the brain may account for about 31% of bioavailability of the drug[11]. Therefore, it is necessary to investigate the methods that could improve penetration of sunitinib through the blood-brain barrier.

The anti-tumour activity of sunitinib in the therapy of choroidal melanoma has resulted in growing interest in the possibility to increase its penetration into the eye[6,7]. The transport of drugs from the circulating blood to inner parts of the eye is regulated by the blood-ocular barriers: the blood–aqueous humour barrier (BAB) and the blood–retinal barrier (BRB). These barriers significantly restrict the ocular availability of drugs administered systemically as well as prevent the penetration of therapeutic agents applied by periocular routes[12,13]. For this reason, ocular melanoma is a malignant cancer for which there are not too many treatment options at the moment. Therefore, it would be desirable to achieve greater concentrations of the drug in the aqueous humour in the cancer therapy.

Many studies have shown that the adjustment of anti-cancer treatment to the circadian rhythm reduces the toxicity of chemotherapeutic agents in healthy tissues and significantly improves their efficacy in tumour cells[14-16]. Drugs targeted at proliferation pathways or interfering with the cell cycle are significantly more potent if they are administered at a specific time of the day, when tumour cell proliferation reaches the highest activity. This is the most important premise and aim of the anti-cancer chronotherapy.

However, the implementation of circadian-based chemotherapy is a challenging issue due to the fact that cancer patients may exhibit alterations in the regulations of circadian rhythms[17].

The circadian timing system controls and regulates the pharmacokinetics of drugs and it determines the extent of molecular and cellular response to them[17]. Currently, there is an increasing need to evaluate the influence of the time of the day on the efficacy and toxicity of new anticancer drugs. So far there have been only a few studies on the chronopharmacokinetics of TKIs, including sunitinib[18,19]. Additionally, to our best knowledge, there are no available data concerning the influence of the time of administration on the penetration of sunitinib through the blood-brain and blood-ocular barriers.

The authors of the study[18] conducted experiments on rabbits and proved that the drug administered at 8 p.m. caused an increase in the concentrations of sunitinib in the blood, as compared with the drug administered at 8 a.m.. Kloth et al[19] conducted research on mice and observed that after the administration of sunitinib at 4 a.m. and 4 p.m. the concentration and AUC of the drug was greater than after the administration at 8 a.m. and 8 p.m. The trough concentration of sunitinib in the blood of 16 patients who received the drug was lower after the morning administration (8 a.m.) than after administration early in the afternoon (1 p.m.) or in the evening (6 p.m.). Additionally, after the morning administration there were more adverse reactions. Thus, we can expect increased penetration of the TKI into the cerebrospinal fluid and aqueous humour after administration of the drug in the evening.

The aim of the study was to assess the penetration of sunitinib through the blood-brain barrier and the blood-aqueous humour barrier depending on the time-of-day *in vivo* administration.

Materials and Methods

Reagents

Sunitinib was purchased from LGC Standards (Łomianki, Poland). Acetonitrile, methanol, ethyl acetate, acetic acid were of HPLC grade and were purchased from Merck (Darmstadt, Germany). Ammonium acetate and sodium hydroxide were purchased from Sigma-Aldrich (Poznan, Poland). Water used in the mobile phase

was deionised, distilled and filtered before use, through a Millipore Direct-Q® system (Merck, Darmstadt, Germany). Sutent® was purchased (batch number P177H) from Pfizer Trading Polska, Warsaw, Poland.

Animals

Adult male New Zealand rabbits, weighing 2.6-3.6 kg, were used for experiments. All the rabbits were kept in individual metal cages located in the animal laboratory of the University of Medical Sciences, Department and Unit of Clinical Pharmacy and Biopharmacy. They were acclimatised for two weeks prior to the experiments and were maintained under standard conditions of temperature (23 ± 2°C) and humidity (56-60%) with an alternating 12 h light/dark cycle to accustom them to the conditions of the study (light on: 07:00-19:00 h). The 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, 1,500 JU vitamin D_3, 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 the Poznan University of Medical Sciences.

Evaluation of Sunitinib and SU12662 Pharmacokinetics

48 rabbits were divided into two groups: I – receiving sunitinib in the morning (8 a.m.), and II – receiving sunitinib in the evening (8 p.m.). Sunitinib was administered orally (*p.o.)* at a single dose of 25 mg (suspended in 1 mL of normal saline). The absence of the drug dosage per kg of the rabbit's body weight resulted from the application of a constant daily dose of sunitinib to the patients. The serum, CSF and AH samples were taken from two rabbits at particular time points. The animals were premedicated with ketamine *i.m.* (50 mg/kg) (Bioketan 100 mg/mL, Vetoquinol Biowet, Gorzów Wielkopolski, Poland) and xylazine *i.m.* (10 mg/kg) (Vetaxyl 20 mg/mL, VET-ARGO, Lublin, Poland). After the onset of anaesthesia the rabbits were placed in a lateral recumbent position to ensure the patency of airways. Then an 18 Ga catheter (Becton Dickinson Polska, Warsaw, Poland) was inserted into the central auricular artery in order to collect a blood sample. The hair above the neck skin was removed and the skin was disinfected. The rabbit's 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 NV, Leuven, Belgium) was inserted gently in the midline at 90 degrees into the vertebral column, and the CSF was collected into polypropylene tubes (Sarstedt, Nümbrecht, Germany). No syringe aspiration was applied. Then, the fine needle with the insulin syringe (Polfa Lublin, Lublin, Poland) were inserted into the anterior chamber of the eye and the AH was gently aspirated. The total volume of AH was collected from both eyes of the rabbits and transferred into polypropylene tubes. After the collection of blood, CSF and AH the animals were euthanised with 1 mL/kg of morbital (sodium pentobarbital 133,3 mg/mL, pentobarbital 26,7 mg/mL; Biowet Puławy, Puławy, Poland) administered intravenously into the marginal ear vein.

Blood samples (3 mL), CSF samples (250-1000 mL), AH (250-500 mL) for sunitinib and SU12662 assays were collected before administration of the drug and 1, 2, 4, 6, 7, 8, 9, 10, 12, 24, 30 hours following the administration. The blood samples were transferred into heparinised tubes (Medlab Products, Raszyn, Poland) and they were centrifuged at 4,000 rpm for 8 min at 4°C. Next the plasma was transferred to polypropylene tubes and stored at -20°C until analysis. The CSF and AH samples were stored under the same conditions as the blood plasma samples until analysis.

The measurement of sunitinib concentration in the blood plasma, CSF and AH was made by means of the high-performance liquid chromatography method (HPLC) with UV detection, which was a modification of the method developed by Faivre et al[20]. Plasma samples of 1,000 μL were diluted with 1,000 μL of sodium hydroxide (1M/L) and subsequently extracted with 3,000 μL of ethyl acetate. During a single extraction the samples were shaken for 10 minutes at 1,500 rpm and then centrifuged at 4,000 rpm for 10 min at 4°C. The extracted solution of 2,500 μL was evaporated to dryness under nitrogen steam at 40°C for approximately 20 min. CSF and AH samples were prepared in the same way using proportionally smaller amounts of the reagents.

The residue was reconstituted in 140 μL of the mobile phase. The resulting solution was placed in 150 μL HPLC microvials (Waters, Warsaw, Poland) and a volume of 50 μL was injected into the HPLC column. 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® C8 column (250 mm´4.6 mm, 5.0 mm particle size) (Waters, Warsaw, Poland). 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 minutes.

The plasma samples were analysed against the calibration curve, obtained from calibration standards prepared in blank rabbit's plasma. The lower limit of quantification (LLOQ) for sunitinib was 10.0 ng/mL. Intra- and inter-day precision and accuracy of the low quality control (QC) (25.0 ng/mL), medium QC (125.0 ng/mL), and high QC (200.0 ng/mL) were well within the acceptable limit of 10% coefficient of variation (CV%). The calibration for sunitinib was linear and ranged from 10.0 to 250.0 ng/mL (r=0.998).

The LLOQ for SU12662 was 1.0 ng/mL. Intra- and inter-day precision and accuracy of the low QC (2.5 ng/mL), medium QC (25.0 ng/mL), and high QC (45 ng/mL) were within the acceptable limit of 10% coefficient of variation (CV%). The calibration for SU12662 was linear in the range 1.0-50.0 ng/mL (r = 0.998).

The CSF and AH samples were analyzed against the calibration curve obtained from calibration standards prepared in ultra-purified water. The LLOQ for sunitinib was 0.25 ng/mL. Intra- and inter-day precision and accuracy of the low QC (0.75 ng/mL), medium QC (5.0 ng/mL) and high QC (8.0 ng/mL) were within the acceptable limit of a 10% coefficient of variation (CV%). The linearity of the method was proved for the range of 0.25-10.0 ng/mL (r = 0.999).

Pharmacokinetic Analysis

Pharmacokinetic parameters were estimated with non-compartmental methods using software (Biokinetica v. 4.1, Biokinetica SA Poland; Phoenix™ WinNonlin® v. 6.3; Certara L.P. USA). The following pharmacokinetic parameters were calculated for sunitinib in the blood, CSF and AH: area under the concentration-time curve from zero to the time of the last measurable concentration (AUC_{0-t}), maximum observed concentration (C_{max}), time to the first occurrence of C_{max} (t_{max}), area under the first moment curve ($AUMC_{0-t}$), mean residence time from zero to the time of the last measurable concentration (MRT_{0-t}). The same pharmacokinetic parameters were calculated for SU12662 in the blood.

Results

All the data were expressed as the mean value of ± standard deviation (SD). The groups did not differ significantly in body mass.

The plasma C_{max} and AUC_{0-t} values in group II receiving sunitinib in the evening (8 p.m.) was 61.4% and 49.6% higher than in group I receiving sunitinib in the morning (8 a.m.). Similarly, the aqueous humour C_{max} and AUC_{0-t} values for sunitinib in group II was greater than in group I (76.9% and 76.6%, respectively). There were also differences in cerebrospinal C_{max} and AUC_{0-t} values for sunitinib in the analysed groups (4.4 vs. 5.7 ng/mL and 66.3 *vs.* 55.5 ng´h/mL, respectively).

The plasma C_{max} and AUC_{0-t} values for SU12662 in group II were 43,4% and 49,9% higher than in group I. There were no measurable concentrations of SU12662 in rabbits' AH and CSF.

Table I shows all pharmacokinetic parameters calculated for both groups. The coefficients of sunitinib penetration through the blood-aqueous humour barrier the blood-brain barrier were estimated from the ratio of sunitinib concentration in the AH and in CSF over the plasma concentration (concentration (C_{AH}/C_{plasma}, C_{CSF}/C_{plasma} respectively). The C_{AH}/C_{plasma} and C_{CSF}/C_{plasma} ratios in group I (0.022 and 0.048, respectively) and in group II (0.024 and 0.023, respectively) were very low.

Figure 1 shows the concentrations of sunitinib in the blood plasma in both groups. Figures 2 and Figure 3 show the concentrations of sunitinib in the rabbits' AH and CSF, respectively.

Discussion

Metastatic brain tumours are observed in 3.9-24% of patients with renal cell carcinoma[21], so they are a frequent complication in this neoplastic disease. The treatment of brain metastases is still one the most challenging issues in oncology. In spite of available treatment options, the prognosis for patients with brain metastases remains poor. One of the main causes of this fact is the limited ability of chemotherapeutic agents to penetrate through the blood-brain barrier (BBB)[22,23]. The intact BBB, formed by endothelial cells lining the brain microvessels and supported by astrocytes and pericytes, is permeable by diffusion only for small lipophilic molecules

Table I. Pharmacokinetic parameters for sunitinib following a 25 mg single oral dose of sunitinib.

Pharmacokinetics parameters	$I_{8a.m.}$	$II_{8p.m.}$
Plasma (sunitinib)		
AUC_{0-t} (ng × h/mL)	2051.8	3069.3
C_{max} (ng/mL)	118.2	190.8
t_{max} (h)	8.0	7.0
MRT_{0-t} (h)	13.9	13.5
$AUMC_{0-t}$ (ng × h^2/mL)	28601.7	41436.4
Aqueous humor (sunitinib)		
AUC_{0-t} (ng × h/mL)	43.2	76.3
C_{max} (ng/mL)	2.6	4.6
t_{max} (h)	6.0	12.0
MRT_{0-t} (h)	13.8	14.9
$AUMC_{0-t}$ (ng × h^2/mL)	595.2	1138.2
Cerebrospinal fluid (sunitinib)		
AUC_{0-t} (ng × h/mL)	55.5	66.3
C_{max} (ng/mL)	5.7	4.4
t_{max} (h)	2.0	7.0
MRT_{0-t} (h)	12.3	16.5
$AUMC_{0-t}$ (ng × h^2/mL)	683.7	1095.3
Aqueous humor/plasma (sunitinib)		
AUC_{0-t} (ng×h/mL)	0.021	0.025
C_{max} (ng/mL)	0.022	0.024
Cerebrospinal fluid/plasma (sunitinib)		
AUC_{0-t} (ng×h/mL)	0.027	0.022
C_{max} (ng/mL)	0.048	0.023
Plasma (SU12662)		
AUC_{0-t} (ng×h/mL)	356.8	534.8
C_{max} (ng/mL)	22.6	32.4
t_{max} (h)	8	7
MRT_{0-t} (h)	14.8	13.6
$AUMC_{0-t}$ (ng × h^2/mL)	5270.6	7274.2

$I_{8a.m.}$, the group with administration of sunitinib at 8a.m.; $II_{8p.m.}$, the group with administration of sunitinib at 8p.m.; AUC_{0-t} – area under the concentration-time curve from zero to the time of last measurable concentration; C_{max} – maximum observed plasma concentration; t_{max} – time to reach maximum concentration; MRT_{0-t} – mean residence time from zero to the time of last measurable concentration; $AUMC_{0-t}$ – area under the first moment curve.

(molecular weight < 450Da)[24,25]. Thus, most cytotoxic drugs, which are large hydrophilic molecules, are prevented from entering the brain and remain not effective in the treatment of CNS tumours or metastases.

TKIs are small compounds with a molecular weight of about 500 Da, but their penetration through the BBB is low. As far as sunitinib is concerned, its transport is limited by the activity of ATP-binding cassette (ABC) transporters – P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP)[26-38]. P-gp is a membrane protein present in the brain microvessel endothelial cells,

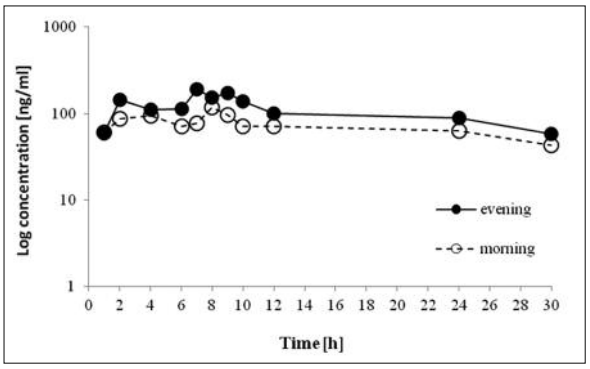

Figure 1. Sunitinib plasma concentration-time profiles following a 25 mg single oral dose of sunitinib administered to rabbits in the morning (8 a.m.) and in the evening (8 p.m.) (arithmetic mean).

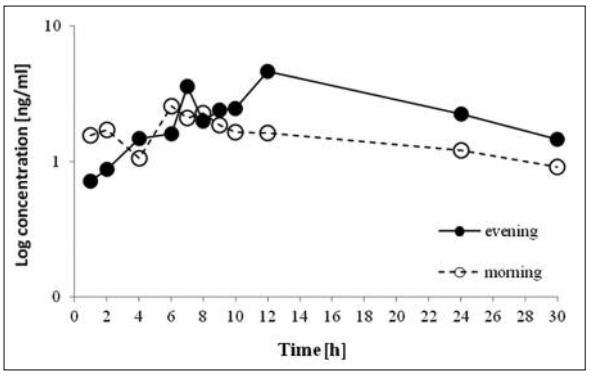

Figure 2. Sunitinib aqueous humour concentration-time profiles following a 25 mg single oral dose of sunitinib administered to rabbits in the morning (8 a.m.) and in the evening (8 p.m.) (arithmetic mean).

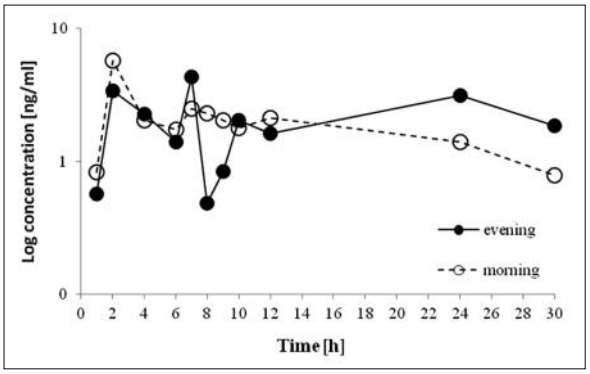

Figure 3. Sunitinib cerebrospinal concentration-time profiles following a 25 mg single oral dose of sunitinib administered to rabbits in the morning (8 a.m.) and in the evening (8 p.m.) (arithmetic mean).

that form the BBB. It actively extrudes its substrates from the cells back into the bloodstream and in this way it limits the entry of many drugs into the CNS[29]. It was shown that together with ABCG2, P-gp effectively restricts the penetration and brain accumulation of sunitinib. However, the application of elacridar, a selective inhibitor of both transporters, to mice, enabled a multiple increase in the concentration of sunitinib in the animals' brains[26].

The blood-aqueous humour barrier (BAB) impedes the transport of drugs to the posterior segment of the eye. It comprises the vascular endothelium of the iris and the non-pigmented ciliary epithelium. It is known that P-gp is expressed in the iris, ciliary muscle, and ciliary non-pigmented cells and the available data clearly confirm the importance of P-gp in regulating the drug flux between the blood and intraocular tissues[12]. As sunitinib is a substrate of P-gp, the P-gp activity can affect its penetration to the aqueous humour.

Although, there are numerous studies demonstrating the influence of circadian rhythms on the efficacy and safety of chemotherapeutic agents[14-16], so far little attention has been paid to the chronobiology of the processes of transport through the blood-brain barrier and through the blood-aqueous humour barrier. Due to the encouraging results of the efficacy of sunitinib in the treatment of brain metastases and ocular melanoma, it is worth to investigate if the time of sunitinib administration during the day will influence the brain and ocular exposure to the drug.

In our study, in the rabbits that received the drug in the evening (8 p.m.) the blood plasma C_{max} and AUC_{0-t} values of sunitinib were respectively 61.4% and 49.6% greater than in the animals to which the drug was administered in the morning. The higher values of sunitinib AUC in the animals from group II prove greater exposure to the drug. These results are consistent with the results obtained in the previous study conducted by the Authors[18]. The AUC_{0-t} values for sunitnib in the AH and in the CSF were also elevated in the rabbits that received the drug in the evening (76.6% and 19.5%, respectively). However, the degree of penetration through the blood-aqueous humour barrier and blood-brain barrier was low and similar in both groups (BAB: 2.2% *vs*. 2.4%, BBB: 4.8% *vs*. 2.3%).

The increase in the exposure to sunitinib in the blood plasma, AH and CSF may be explained by circadian variations in the activity of P-gp, which is responsible for the absorption of sunitinib in the intestine and its active transport through the blood-aqueous humour and blood-brain barriers. Ando et al[30] conducted a study on day-night profiles of P-gp mRNA and protein levels in the peripheral tissues of mice. They demonstrated that in the mouse intestine the levels of P-gp expression and its function exhibited a 24-h rhythmicity, with a peak observed in the terminal part of the light phase and the minimum level occurring at the onset of the light phase. Okyar et al[31] found that the intestinal activity of P-gp increased during rats' night-time activity. The authors of another study showed the significant influence of the time of administration on the P-gp-mediated transport in the rat's brain. It was substantially increased during the animals' active period[32]. Recent studies conducted on monkeys question the utility of the results obtained with rodents, raising concerns that it would be difficult to predict circadian changes of the drug pharmacokinetics in diurnal humans on the basis of data achieved from nocturnal rodents[33]. However, due to the fact that our animals had the circadian clock synchronised with the human activity, we can conclude that our findings confirm the data from the abovementioned studies on animals, and they might reflect variations observed in humans. Nonetheless, this hypothesis needs to be examined in further studies on humans.

Conclusions

The exposure to sunitinib in the rabbits' plasma, AH and CSF was greater after the evening administration (8 p.m.) than after the morning administration (8 a.m.), but the degree of penetration of sunitinib through the blood-aqueous humour and blood-brain barriers was very low (< 5%) and comparable in both groups..

Conflict of Interest

The Authors declare that there are no conflict of interests.

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