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Original research article

The pharmacokinetics and hypoglycaemic effect of sunitinib in the diabetic rabbits

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ABSTRACT

Background: Diabetes is one of the most common metabolic diseases in the world, which may influence changes in the pharmacokinetics and pharmacodynamics of drugs. Sunitinib is a tyrosine kinase inhibitor (TKI) broadly used for treatment of numerous cancers, which exhibits the side hypoglycaemic effect. The aim of the study was a comparison of concentrations and pharmacokinetics of sunitinib after a single administration in rabbits with hyperglycaemia and normoglycaemia (control group). Additionally, the effect of sunitinib on glucose levels was investigated.

Methods: The research was carried out on a control group ($n = 6$) and a group of rabbits with diabetes ($n = 6$). The rabbits were treated with sunitinib in the oral dose of 25 mg. Plasma concentrations of sunitinib and its metabolite (SU12662) were measured with validated HPLC method with UV detection.

Results: The comparison of the sunitinib C_{max} and $AUC_{0-\infty}$ in the diabetic group with the control group gave the ratios of 1.63 [90% confidence interval (CI) [1.59; 1.66] and 2.03 [1.97; 2.09], respectively. Statistically significant differences between the analyzed groups were revealed for C_{max} ($p = 0.006$), $AUC_{0-\infty}$ ($p = 0.0088$), and AUC_{kel} ($p = 0.009$). The maximum glycaemia drop of 14.4–69.6% and 15.4–33.5% was observed in the diabetic animals and in the control group, respectively. The glycaemia values returned to the initial values in 24 h after the administration of the drug.

Conclusions: The research proved the significant influence of diabetes on the pharmacokinetics of sunitinib and it confirmed the hypoglycaemic effect of the TKI in diabetic rabbits and in normoglycaemia.

Introduction

Diabetes is an epidemic of the 21st century. The number of diabetic patients is still growing. When discussing diabetes it is necessary to take numerous complications of the disease into consideration (neuropathy, nephropathy, retinopathy), which may result in a change in the pharmacokinetics and pharmacodynamics of simultaneously administered drugs. Changes at the absorption stage result from reduced gastric mucosal blood flow and delayed gastric emptying time, at the distribution stage – from non-enzymatic glycation of albumin, at the elimination stage – from changed regulation of hepatic enzymes and nephropathy [1]. At present we have numerous groups of drugs with different mechanisms of action, which enable successful treatment of diabetes. However, hypoglycaemia is a serious adverse reaction in the anti-diabetic therapy. It may be the consequence of a wrong dose of a hypoglycaemic drug as well as the effect of drug interactions [2,3]. Hypoglycaemia may also be a side effect of another drug [3]. This effect can be observed in tyrosine kinase inhibitors (TKIs) [4] in patients with normoglycaemia and diabetes [5]. One of the TKIs that reduces the concentration of glucose in the blood is sunitinib [6].

Sunitinib demonstrates good anti-tumour activity by inhibiting platelet-derived growth factor receptors (PDGFRα and PDGFRβ), vascular endothelial growth factor receptors (VEGFR1, VEGFR2, VEGFR3), stem cell factor receptors (c-Kit), Fms-like tyrosine

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kinase 3 receptors (FLT3), colony-stimulating factor receptors (CSF-1R), glial cell-derived neurotrophic factor receptors (RET). Blocking so many signalling routes must undoubtedly influence the multidirectional effect of the drug. Therefore, sunitinib became the drug of choice for the treatment of metastatic renal cell carcinoma (mRCC), gastrointestinal stromal tumours (GIST), neuroendocrine tumours, breast cancer and lung cancer [7,8].

The use of sunitinib is becoming more common. Therefore it seems to be important to know whether diabetes has influence on the concentrations and pharmacokinetics of the kinase inhibitor.

The aim of the research was an analysis of the pharmacokinetics and hypoglycemic effect of sunitinib in rabbits with diabetes. We performed a National Library of Medicine's bibliographic database (MEDLINE®) search and found no evidence in the literature regarding the effects of diabetes on the pharmacokinetics of sunitinib.

Materials and methods

Reagents

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

Animals

Adult New Zealand rabbits of either sex, weighing 2.8–4.7 kg, were used for experiments. The rabbits were divided into two groups (6 animals in each). All rabbits were kept in individual metal cages located in the animal laboratory of Poznan 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 ± 2 °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 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 Medical University of Poznan. The animals were randomized to appropriate group by coin toss. The number of rabbits in the groups was based on the researchers' earlier experiments [9–11].

Induction of diabetes by alloxan injection

A single dose of alloxan (90 mg/kg) was injected into the lateral ear vein to induce diabetes mellitus. Alloxan (90 mg/ml) was freshly dissolved in sterile saline. Control rabbits received an equivalent amount of sterile saline. To prevent hypoglycemia, 10 ml of glucose 5% was injected intravenously (*i.v.*) after the alloxan and drinking water supplemented with 10% glucose for the first 24 h after the alloxan injection. The fasting blood sugar levels of each of the rabbits were checked everyday from the 5th day with an autoanalyzer (AccuCheck Active®) glucose kit. The rabbits with blood glucose level ≥ 250 mg/dl were considered to be diabetic and were used in the experiment [12]. The percentage reduction of the glucose levels of the rabbits was calculated using the formula:

$$\%\ \text{reduction}_{\text{glucose}} = \frac{(V_0 - V_t) \cdot 100}{V_0}$$

where V_0 – glucose concentration at zero hour and V_t – glucose concentration at hour with maximum reduction.

Evaluation of sunitinib and SU12662 pharmacokinetics

The rabbits were divided into two groups: the rabbits with diabetes receiving sunitinib (I), and the control group receiving sunitinib (II). Sunitinib was administered orally (*p.o.*) at the single dose of 25 mg (suspended in 10 ml of normal saline). The absence of drug dosage per kg of the rabbit's body weight resulted from the application of a constant daily dose of sunitinib to the patients [13]. Blood samples (3 ml) for sunitinib and SU12662 assays were collected before and 0.50, 1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, 96 h following drug administration. The blood samples were transferred into heparinised tubes and they were centrifuged at 4000 rpm for 10 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 was made by means of the high-performance liquid chromatography method (HPLC) with UV–vis detection, which was a modification of the method developed by Faivre et al. [14]. Plasma samples of 1000 μl were diluted with 1000 μl of sodium hydroxide (0.1 M/l) and subsequently extracted with 3000 μl of ethyl acetate. During single extraction samples was shaken out for 10 min at 1500 rpm and then centrifuged at 4000 rpm for 10 min at 4 °C. The extracted solution of 2500 μl was evaporated to dryness under nitrogen steam at 40 °C for approximately 20 min. The residue was reconstituted in 140 μl of mobile phase. The resulting solution was introduced into 150 μl HPLC microvials and a volume of 50 μl was injected onto 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 μm particle size) (Waters®). The column temperature was maintained at 40 °C, the UV–vis detection wavelength was set at 431 nm, and the injection volume was 50 μl. 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. Intra- and inter-day precision and accuracy of the LLOQ, 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 ng/ml (r = 0.999), for SU12662 in the range 1.0–50.0 ng/ml (r = 0.998).

Pharmacokinetics analysis

Pharmacokinetic parameters were estimated by non-compartmental methods using validated software (Phoenix™ WinNonlin® v. 6.3; Certara L.P. USA). The following pharmacokinetic parameters were calculated for sunitinib: elimination rate constant (k_{el}), area under the plasma concentration–time curve from time zero to infinity (AUC_{0-inf}), area under the plasma concentration–time curve from zero to the time of last measurable concentration (AUC_{0-t}), area fraction under the curve between first and last sampling point used for k_{el} calculation (AUC_{kel}); maximum observed plasma concentration (C_{max}), time to first occurrence of C_{max} (t_{max}), half-life in elimination phase ($t_{1/2kel}$), clearance (Cl), volume of distribution (V_d), area under the first moment curve ($AUMC_{0-t}$), mean residence time (MRT). The pharmacokinetic endpoints for SU12662 were AUC_{0-inf}, AUC_{0-t}, C_{max}, and t_{max}.