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Short communication

# The alteration of pharmacokinetics of erlotinib and OSI420 in type 1 diabetic rabbits

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ABSTRACT

*Background:* Alterations in blood glucose levels observed in diabetes, may change the pharmacokinetics of co-administered drugs and in consequence, the efficacy and safety of therapy. Many oncological patients are diabetics and it is important to determine the interaction of anticancer drugs with this chronic disease. Erlotinib is a tyrosine kinase inhibitor (TKI), approved for the treatment of patients with non-small-cell lung cancer and pancreatic cancer in combination with gemcitabine. The aim of the study was to investigate the influence of the diabetes on the pharmacokinetics of erlotinib in rabbits. Additionally, the effect of erlotinib on glucose levels was examined.

*Methods:* The pharmacokinetics of erlotinib was studied in healthy rabbits ($n = 6$, control group) and type 1 diabetic rabbits ($n = 6$, diabetic group). Erlotinib was administered in a single oral dose of 25 mg. Plasma concentrations of erlotinib and its metabolite (OSI420) were measured with the validated method.

*Results:* The plasma concentrations of erlotinib and OSI420 were markedly increased in diabetic rabbits. Statistically significant differences between the groups were revealed for almost all analysed pharmacokinetic parameters for erlotinib and OSI420. The maximum glycaemia drop of 7.7–33.5% was observed in the diabetic animals, but no significant changes in glucose concentration were observed in the control group.

*Conclusions:* The research proved the significant influence of diabetes on the pharmacokinetics of erlotinib and OSI420. Due to higher exposure to erlotinib, there may be an increased risk of adverse drug reactions in diabetic patients. Therefore, in some cases lower doses of the drug should be considered.


## Introduction

Erlotinib is an inhibitor of the epidermal growth factor receptor (EGFR) approved for the treatment of advanced non-small cell lung cancer (NSCLC) as a single agent, and metastatic pancreatic cancer in combination with gemcitabine [1]. Erlotinib is metabolised by CYP3A4/3A5 and, to a lesser extent, by CYP1A1/1A2 isoenzymes, to the active metabolite desmethyl erlotinib (OSI420), which subsequently undergoes oxidation and glucuronidation. Extrahepatic metabolism by CYP3A4 in the intestine, CYP1A1 in the lung, and CYP1B1 in the tumour tissue, also potentially contribute to the metabolic clearance of the drug. Erlotinib and OSI420 are considered to be equipotent in inhibiting EGFR tyrosine kinase activity [2]. Erlotinib is well absorbed after oral administration (60%), and its bioavailability is approximately 100% when given with food. Peak plasma levels occur around 4 h postdose and the median elimination half-life ($t_{1/2k_{el}}$) is 36 h. The drug is highly protein bound (approximately 93%) to albumin and alpha-1-acid glycoprotein (AAG).

Numerous studies on animals and humans have proved the relationship between hyperglycaemia and pharmacokinetic parameters of drugs. Diabetes affects the expression of many isoforms of cytochrome P450. Baek et al. [3] proved increased CYP2E1 activity, which enhanced the concentration of chlorzoxazone metabolite – 6-hydroxy chlorzoxazone (OH-CZX) in rats with experimentally induced diabetes. Due to higher activity of

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CYP2E1, CYP1A1/2 and CYP3A1/2 isoenzymes in diabetic animals, the clearance of many drugs were faster and AUC values were reduced, when compared with the control group [4]. Increased CYP1A1/2 activity was also revealed for the 5-FU metabolism in diabetic rats (a decrease in AUC and increase in 5-FU clearance) [5]. However, CYP3A4 activity may be reduced in diabetes [4] and this observation has been proved for sunitinib [6].

Approximately 8–18% of cancer patients suffer from diabetes mellitus [7]. Among patients with advanced NSCLC, about 8% are diabetics [8]. Thus, erlotinib is very likely to be applied to this group of patients.

The aim of the research was an analysis of the pharmacokinetics and hypoglycaemic effect of erlotinib 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 erlotinib.

## Materials and methods

### *Reagents*

Erlotinib and OSI420 were purchased from LGC Standards (Łomianki, Poland), ammonium acetate, alloxan and acetic acid were purchased from Sigma–Aldrich. Methanol and acetonitrile were purchased from Merck. Water used in the mobile phase was deionized, destilled and filtered through a Millipore Direct-Q® system (Merck, Darmstadt, Germany) before use. Tarceva® were purchased (batch number M1000B01) from Roche Polska (Warsaw, Poland).

### *Animals*

All experimental protocols for this study were reviewed and approved by the Local Ethics Committee. Adult New Zealand rabbits of either sex, weighing 2.8–4.7 kg, were used for experiments. All the animals were kept in individual metal cages at the same temperature (23 ± 2 °C), humidity (56–60%) and under light/dark cycles (12 h). The rabbits were provided with 100 g of commercial pelleted diet (Labofeed KB®, Kcynia, Poland) and tap water *ad libitum*.

### *Induction of diabetes by alloxan injection*

A single dose of alloxan (90 mg/kg), freshly dissolved in 0.9% NaCl solution, was injected into the lateral ear vein to induce diabetes mellitus. The fasting blood glucose levels of each rabbit were checked once daily from the 5th day with an autoanalyzer glucose kit (AccuCheck Active®, Roche Diabetes Care Polska, Warsaw, Poland). The rabbits with blood glucose level ≥250 mg/dl were considered to be diabetic and were used in the experiment. The percentage reduction of the glucose levels of the rabbits was calculated using the formula:

$$\%reduction_{glucose} = \frac{(V_0 - V_t) \times 100}{V_0}$$

where $V_0$, glucose concentration at 0 h; and $V_t$, glucose concentration at hour with maximum reduction.

### *Evaluation of erlotinib and OSI420 pharmacokinetics*

The rabbits were divided into two equal groups ($n = 6$): the rabbits with diabetes receiving erlotinib (I), and the control group receiving erlotinib (II). Erlotinib was administered orally (*po*) at the single dose of 25 mg (suspended in 1 ml of 0.9% NaCl solution). The absence of drug dosage per kg of the rabbit's body weight resulted from the application of a constant daily dose of erlotinib to the patients. Blood samples (2 ml) for erlotinib and OSI420 assays were collected before and 0.50, 1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 24, 48 h following drug administration.

The measurement of erlotinib and OSI420 concentration in the blood plasma was made by means of the high-performance liquid chromatography (HPLC) method with UV detection, which was a modification of the method developed by Faivre et al. [9]. The lower limit of quantification for erlotinib and OSI420 were 10.0 ng/ml and 2.5 ng/ml, respectively. The calibration curve was linear in the range of 10.0–1000 ng/ml ($r = 0.999$) for erlotinib and in the range of 2.5–550.0 ng/ml ($r = 0.997$) for OSI420.

### *Pharmacokinetics analysis*

Pharmacokinetic parameters were estimated by non-compartmental methods using validated software Phoenix® WinNonlin® 6.4 (Certara L.P., Princeton, USA).

### *Statistical analysis*

The differences in the values of pharmacokinetic parameters were analysed by means of Student *t*-test using PROC TTEST in SAS (SAS Institute Inc., 2002-2010). The SAS System for Windows version 9.3. (Cary, NC 27513-2414, USA). The differences that generated *p*-values $<0.05$ were considered statistically significant.

## Results

All the data were expressed as the mean value ± standard deviation (SD). Both the groups of rabbits did not differ significantly in terms of body mass. The high values of the coefficients of variations (CV%) indicate the large variability in pharmacokinetic parameters between rabbits (Table 1).

$C_{max}$ of erlotinib and OSI420 were achieved within 2–5 h. There were no statistically significant differences in erlotinib and OSI420 $t_{max}$ ($p = 0.4288$, $p = 0.1041$, respectively). The arithmetic mean plasma concentrations of erlotinib and OSI420 in the analysed groups after administration of erlotinib are shown in Figs. 1 and 2, respectively.

In the diabetic rabbits the exposure to erlotinib was higher than in the nondiabetic (control) group, as evidenced by increased values of $C_{max}$ ($p \leq 0.0001$) and $AUC_{0-t}$ ($p = 0.0003$). The diabetic group was characterised by significantly prolonged $t_{1/2k_{el}}$ ($p = 0.0273$), as well as reduced $V_{ss}$ ($p = 0.0003$), Cl ($p = 0.0013$) and $k_{el}$ values ($p = 0.0112$).

The diabetic and the nondiabetic rabbits exhibited also alterations in $C_{max}$ and $AUC_{0-t}$ of erlotinib metabolite (OSI420). The mean OSI420 $C_{max}$ and $AUC_{0-t}$ were higher in the diabetic group than in the control group. The differences between the analysed groups in OSI420 $C_{max}$ and $AUC_{0-t}$ were statistically significant ($p = 0.0034$, $p = 0.0055$, respectively).

The diabetic rabbits experienced drop in the glycaemia, ranged from 7.7 to 33.5%. There were no significant changes in blood glucose levels in the nondiabetic group. The arithmetic mean glucose plasma concentration–time profiles in both groups of rabbits are presented in Fig. 3.

## Discussion

Diabetes has very complex influence on drug pharmacokinetics. Studies on animals with experimentally induced diabetes and on diabetic patients proved reduced CYP3A4 activity and increased expression of CYP2E1 and CYP1A2 [4]. Additionally, the increase in the drug concentration in diabetes is caused by glycation of