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Pharmacokinetic/pharmacodynamic modeling of etoposide tumor growth inhibitory effect in Walker-256 tumor-bearing rat model using free intratumoral drug concentrations

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ABSTRACT

The purpose of this study was to establish a population pharmacokinetic/pharmacodynamic (PK/PD) model linking etoposide free tumor and total plasma concentrations to the inhibition of solid tumor growth in rats. Walker-256 tumor cells were inoculated subcutaneously in the right flank of Wistar rats, which were randomly divided in control and two treated groups that received etoposide 5 or 10 mg/kg i.v. *bolus* every day for 8 and 4 days, respectively, and tumor volume was monitored daily for 30 days. The plasma and intratumoral concentrations-time profiles were obtained from a previous study and were modeled by a four-compartment population pharmacokinetic (popPK) model. PK/PD analysis was conducted using MONOLIX v.4.3.3 on average data and by mean of a nonlinear mixed-effect model. PK/PD data were analyzed using a modification of Simeoni Tumor Growth Inhibition (TGI) model by introduction of an E_{max} function to take into account the concentration dependency of $k_{2variable}$ parameter (variable potency). The Simeoni TGI-E_{max} model was capable to fit schedule-dependent antitumor effects using the tumor growth curves from the control and two different administered schedules. The PK/PD model was capable of describing the tumor growth inhibition using total plasma or free tumor concentrations, resulting in higher k_{2max} (maximal potency) for free concentrations (25.8 $mL \cdot \mu g^{-1} \cdot day^{-1}$ - intratumoral vs. 12.6 $mL \cdot \mu g^{-1} \cdot day^{-1}$ total plasma). These findings indicate that the plasma concentration may not be a good surrogate for pharmacologically active free tumor concentrations, emphasizing the importance of knowing drug tumor penetration to choose the best antitumor therapy.

1. Introduction

In the past decades, the application of pharmacokinetic/pharmacodynamic (PK/PD) modeling in the drug development process has increased substantially and has received more attention from the industry and regulatory agencies (Garnett et al., 2011; Gobburu, 2010; Jonsson et al., 2011). The PK/PD modeling using preclinical and clinical data has become a useful alternative for rational development of new drugs through early understanding of dose–response relationship and has enabled the optimization of dosing regimens for existing approved drugs, respectively (Bender et al., 2015; Friberg, 2003; Van Kesteren et al., 2003).

Because anticancer agents usually have a narrow therapeutic window, PK/PD models can be extremely useful in oncology guiding the selection of adequate doses that improve treatment efficacy and reduce toxicity (Mould et al., 2015). PK/PD models developed in oncology have been applied to describe the relation between drug plasma concentration and tumor growth (Ribba et al., 2012; Simeoni et al., 2004), biomarker response (Lindauer et al., 2010; Yamazaki et al., 2008), as well as adverse effects (Friberg et al., 2002; Quartino et al., 2012), using data from animals or humans.

The most usual PD marker in oncology is the tumor growth, where the measurements of the tumor volume are used to construct the time course of growth after administration of anticancer agents (Ribba et al., 2012; Rocchetti et al., 2005; Salphati et al., 2010; Simeoni et al., 2004). The most popular preclinical PK/PD model of tumor growth was developed by Simeoni et al. (2004). This model was primarily

Abbreviations: W256, Walker-256; HPLC-UV, high pressure liquid chromatography-ultraviolet; PK/PD, pharmacokinetic/pharmacodynamic; popPK, population pharmacokinetic; TGI, Tumor Growth Inhibition; NAD, Naïve Average Data; SAEM, stochastic approximation expectation maximization; GOF, goodness-of-fit; VPC, visual predictive check; AUC, area under the curve.

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developed for ranking competing preclinical candidates and was expanded to describe the tumor growth dynamics after administration of drug combinations (Terranova et al., 2013) as well as to predict suitable doses in humans from animal studies (Rocchetti et al., 2007).

The PK data most used to build the PK/PD model in pre-clinical and clinical oncology studies are the plasma concentrations assuming that these are a good surrogate for the drug concentrations reached in the tumor. Nevertheless, linking the effect to drug plasma concentrations can be misleading, since drug delivery into solid tumors is limited due to the heterogeneous microenvironment, with abnormal vascularization, hypoxic areas and high interstitial pressure characteristic of the tumor (Gallo, 2010; Grantab and Tannock, 2012; Wei et al., 2009; Zhou and Gallo, 2005). Drug plasma concentrations are commonly higher than those determined in the tumor as observed previously with epirubicin (Hunz et al., 2007), methotrexate (Sani et al., 2010) and reviewed by Fuso Nerini et al. (2014).

In this scenario, PK models that describe the concentrations in the tumor compartment can provide a better understanding of the drug distribution and drug efficacy helping to optimize dosing schedules. Up to date only a few PK/PD models have related anticancer tumor concentrations and effect, such as the model reported for temozolomide (Zhou et al., 2007), gefitinib (Sharma et al., 2013; Wang et al., 2008, 2009) and paclitaxel (Colin et al., 2014). Furthermore, these studies only investigated drug penetration into brain tumors, demonstrating the need for studies that consider the anticancer distribution to other types of solid tumors.

The anticancer agent etoposide is a topoisomerase II inhibitor used for treating hematopoietic malignancies and different solid tumors, such as small cell lung cancer, breast cancer and Kaposi's sarcoma. Although the systemic PK and PD of etoposide are extensively studied (Slevin, 1991; Toffoli et al., 2001), little is known about its distribution in solid tumors and PK/PD modeling linking its intratumoral concentrations with antitumor effect has not been reported.

In this context, the present study aims to comparatively model the PK/PD relationship between total plasma and free interstitial tumor etoposide concentrations to the tumor growth kinetics observed in a Walker-256 (W256) tumor-bearing Wistar rat model.

2. Materials and Methods

2.1. Chemicals and Reagents

Etoposide (purity ≥98%) and Trypan Blue solution 0.4% were purchased from Sigma-Aldrich (St. Louis, USA). Ethyl alcohol (anhydrous) and formic acid were purchased from Tedia (Fairfield, USA). Ultrapure water was obtained in a Millipore Milli-Q system (Bedford, USA). Polyethylene glycol (PEG) 300, polysorbate 80 and citric acid were acquired from Labsynth (São Paulo, Brazil). Glucose sterile solution was purchased from Basa (Caxias do Sul, Brazil). All other chemicals and reagents used in this study were of pharmaceutical or analytical grade.

Etoposide solution (5 mg/mL) was prepared for intravenous (IV) administration containing 3% citric acid 10%, 25% polyethylene glycol, 7.5% polysorbate 80, 10% ethanol (*v*/*v*) and the final volume was obtained with 5% glucose solution. This formulation is similar to the commercial injectable formulation used in humans (Kaul et al., 1995; Toffoli et al., 2001).

2.2. Animals and Tumor Model

Male Wistar rats (150–200 g) were supplied by the Center for Reproduction and Experimentation of Laboratory Animals (CREAL/UFRGS - Porto Alegre, Brazil) and received food and water ad libitum. Animal procedures were approved by UFRGS Ethical Committee on Animal Use (CEUA/UFRGS, protocol number 22302) and were conducted under standard conditions according Brazilian law (Brazil, 2008) and the guideline on experimental animal care and use (Brazil, 2013).

To obtain the tumor model, W256 carcinosarcoma cells were implanted intraperitoneally (IP) into Wistar rats (1×10^7 viable cells per animal). After 5–7 days of implantation, the ascitic tumor was harvested from the peritoneal cavity and the cell viability was evaluated by Trypan blue exclusion test (Phillips, 1973) using a Neubauer's chamber (Brand, Wertheim, Germany). To produce a solid tumor, 2×10^7 viable cells in 1 mL of phosphate-buffered solution were inoculated subcutaneously into the right flank of the animal. During harvesting and inoculation procedures the animals were anesthetized with a ketamine-xylazine (100–10 mg/kg). After inoculation, the animals were kept on separated in cages (4 rats/cage) in standard conditions of temperature, humidity and 12-h light–dark cycle during the period of treatment.

2.3. Pharmacokinetic Study

The pharmacokinetics of etoposide in W256 tumor-bearing Wistar rats was previously investigated in plasma and tumor (Pigatto et al., 2016). A population PK model (popPK) was developed using MONOLIX v. 4.3.3 (Lixoft, Orsay, France). The popPK model simultaneously described total etoposide concentrations in plasma and free concentrations in two regions of the tumor – center and periphery consisting of four-compartments with a saturable distribution into the tumor compartments and first-order elimination. The system of differential equations for the popPK model is given in Eq. 1:

$$\begin{aligned}
&\frac{dA(1)}{dt} = A(2)\cdot k_{21} + A(3)\cdot k_{31} + A(4)\cdot k_{41} - A(1)\cdot(k_{10}+k_{12}) - \left(\frac{V_{max}\cdot A(1)}{V_1\cdot k_m + A(1)}\right) - \left(\frac{V_{max}\cdot A(1)}{V_1\cdot k_m + A(1)}\right)\\
&\frac{dA(2)}{dt} = A(1)\cdot k_{12} - A(2)\cdot k_{21}\\
&\frac{dA(3)}{dt} = \left(\frac{V_{max}\cdot A(1)}{V_1\cdot k_m + A(1)}\right) - A(3)\cdot k_{31}\\
&\frac{dA(4)}{dt} = \left(\frac{V_{max}\cdot A(1)}{V_1\cdot k_m + A(1)}\right) - A(4)\cdot k_{41}\\
&C_{plasma} = \frac{A(1)}{V_1}\\
&C_{T.periphery} = \frac{A(3)}{V_3}\\
&C_{T.center} = \left(\frac{A(3)}{V_3}\cdot F_p\right) + \left(\frac{A(4)}{V_4}\cdot(1-F_p)\right)
\end{aligned} \tag{1}$$

A covariate model, in which the volume of plasma compartment V_1 is a function of the body weight, was used (Eq. 2):

$$V_{1i} = 0.171 \cdot \left(\frac{BW_i}{0.290}\right)^{0.581} \tag{2}$$

where V_{1i} is the volume of the central compartment for the i-th individual; 0.171 is the *(population)* volume of the central compartment estimated by the popPK model; 0.581 is the exponential scaling factor; BW is animal's individual body weight (kg); and 0.290 is the mean body weight (kg) in the PK group.

For the present PK/PD modeling, two sets of concentrations were used: total plasma concentration and free tissue concentration in the peripheral region of the tumor, because this region has a higher density of viable cancer cells that can be killed by the drug. Etoposide has a relatively short elimination half-life in tumor periphery ($\approx$2.39 h^{-1}) and in plasma ($\approx$1.83 h^{-1}), thus no accumulation was observed with the dose interval applied in the PD study. Total plasma and free peripheral tumor concentration-time profiles for the different treatments investigated in the PD experiments were simulated by fixing the following mean estimates values from the PK model previously described (Pigatto et al., 2016): elimination rate micro-constant from the central compartment (k_{10}) was 1.27 h^{-1}; the distribution rate micro-constants between compartments k_{12}, k_{21}, k_{31} and k_{41} were 2.86 h^{-1}, 2.88 h^{-1}, 3.99 h^{-1}, and 0.216 h^{-1}, respectively; the volume of the tumor periphery compartment (V_3) was 0.112 L; volume of the tumor center compartment V_4 was 2.99 L; maximum transporter velocity from the plasma to tumor (V_{max}) was 0.907 μg·h^{-1}; Michaelis-Menten constant