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A novel pharmacokinetic model based on the complex elimination of monoclonal antibodies for bevacizumab pharmacokinetic study in rabbits

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ABSTRACT

Monoclonal antibodies (mAbs) complex pharmacokinetic (PK) properties including a nonlinear pharmacokinetics and a significant variation in individual PK process cannot be appropriately described by classic PK models, probably derived form a poor understanding of the complex elimination of mAbs. In this study, a novel PK model based on mAbs' complex drug elimination was established. Subsequently, this new model was used to fit bevacizumab plasma concentration data from PK rabbits, and the outcomes of model fitting were compared with those came from a fit with classic models. In addition, the variations existing in the parameters set in the new model were analyzed. As a result, this novel model reasonably described the single-dose PK profiles of bevacizumab in rabbits, and its fitting efficiency was greatly improved compared with those fitted with classic PK models in terms of the weighted residual sum of squares. Moreover, the variations existing in the new model's parameters $C_{A(antibody)}$ and K_0 could reasonably explain the individual variations of bevacizumab's PK profiles. In conclusion, the novel model reasonably explained the elimination of bevacizumab, and exhibited a potential as a useful tool for the PK studies of bevacizumab and other mAbs in practice.

1. Introduction

Antibodies are large proteins utilized by the immune system for an identification and neutralization of foreign objects such as bacteria and viruses. Monoclonal antibody (mAb) preparations, which derive from a single progenitor through cloning the hybridoma cells, are homogeneous with respect to antibody isotype, primary amino acid sequence, affinity, and specificity [1–2]. Due to a specific binding to targets relevant to disease progress, mAbs can offer considerable advantages over small-molecule drugs, by having fewer adverse effects and/or possibly by increasing the efficacy of treatment than conventional therapy [3–4]. In recent years, more and more mAbs have been approved as therapeutic drugs and entered into the clinic for various diseases, such as cancer, inflammatory diseases and hematological disorders [5–7]. In particular, a considerable clinical success has been achieved with mAbs in cancer therapy for its remarkable pharmacological characteristics, such as high potency, limited off-target toxicity, and long serum half-lives [8–9]. Bevacizumab, a recombinant humanized IgG1 antibody, has been widely employed for the treatment of metastatic colorectal cancer and non-small cell lung cancer as a kind of anti-angiogenic agent for inhibiting effects induced by vascular endothelial growth factor (VEGF).

Pharmacokinetic (PK) analyses are essential components of the drug discovery and development process [10–11]. Particularly in early clinical trials, it is of great importance to study PK to serve itself as an "auxiliary biomarker", helpful for the selection of doses in further trials and promotion of clinical rational drug use [12–14]. Traditional PK models including compartment models and Michaelis–Menten (M–M) model were widely used in the PK analyses of various drugs, especially in small-molecule drugs [15–16]. They provided a convenient way to character drugs' properties with model parameters, such as half-life ($T_{1/2}$), maximum concentration (C_{max}), area under the concentration–time curve (AUC), clearance (CL), and so on. However, much more complex PK properties were showed by mAbs compared to those typically associated with small-molecule drugs, and traditional PK models failed to well describe the complex PK process of mAbs. For instance, complex nonlinear PK was encountered frequently by mAbs [17]. The drug exposure or responses did not proportionally vary with dose increasing, and PK parameters such as CL and apparent volume of distribution (V) were not constant in different dosage. Moreover, PK parameters derived from a compartment model fitting and/or an M–M model fitting were significantly varied among patients, in the case they were hard to provide useful information for personalized medicine [18]. For this reason, some new models have been proposed to fit the nonlinear PK of mAbs in recent years. Mager's group has made a successful attempt by building a target-mediated drug disposition (TMDD) model from the perspective of an interaction between antibodies and their targets. The

Abbreviations: ADCC, antibody dependent cellular cytotoxicity; AUC, area under the concentration–time curve; CL, clearance; mAbs, monoclonal antibodies; M–M, Michaelis–Menten; PBS, phosphate-buffered saline; PK, pharmacokinetic; TMB, tetramethylbenzidine; TMDD, target-mediated drug disposition; $T_{1/2}$, half life; V, apparent volume of distribution; VEGF, vascular endothelial growth factor.

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TMDD model deduced the final mathematical model of antibodies through making a comprehensive analysis about the kinetics of targets, antibodies and target-antibody complexes [17,18]. However, the TMDD model consisted of numerous parameters and the value of some parameters were only available through other complicated experiments such as fluorescence-activated cell sorting analysis and so on, thus probably limiting its extensive applications in clinical practice [19].

Monoclonal antibody drugs exhibits much more complex PK properties probably due to significant differences from small-molecule drugs not only in their pharmacological mechanism of action but also in their elimination mechanism [18–20]. Compared to small-molecule drugs, renal elimination is relatively unimportant for mAbs, as its large size prevents efficient filtration through the glomerulus. Specific to its targets such as antigen, receptor, and some protein or polypeptide, mAbs will encounter a target-mediated endocytosis as most kinds of endogenous IgG [20]. Moreover, a phagocytosis of lymphocyte and even some incompletely explained pathways further complicate the elimination of mAbs [19].

In this work, a novel PK model based on the complex elimination of mAbs was established, and its mathematical expressions were also put forward. Subsequently, bevacizumab was selected as a model drug and the new PK model was used to fit the bevacizumab plasma concentration data from pharmacokinetic rabbits.

2. Materials and methods

2.1. PK model based on complex drug elimination

A novel PK model was constructed based on the complex elimination of mAbs. The model diagram is presented in Fig. 1. When mAbs were firstly administrated, a sharp decrease in concentration occurred due to a specific binding to pre-existing targets. The rest of mAbs were then eliminated through two main pathways. On the one hand, a degradation and metabolism of protein and/or a phagocytosis of lymphocyte could induce mAbs' concentration decrease, due to mAbs were kinds of protein or peptides and they would encounter a general metabolism in vivo like most kinds of proteins or peptides [19,21–22]. This action could happen all the time and it was a concentration-depended process to some extent, so that the apparent elimination rate of this pathway was assumed to be first-order. On the other hand, most targets were endogenous and generated all the time, and a continuous bind with newly generated targets would trigger antibody dependent cellular cytotoxicity (ADCC) and/or complement activity to induce mAbs' another pathway of elimination [23–26]. The rate of this pathway was assumed to be zero-order because targets' zero-order generation was the rate-limiting step in this pathway. A superposition of a first-order process and another zero-order process complicated in vivo elimination of mAbs.

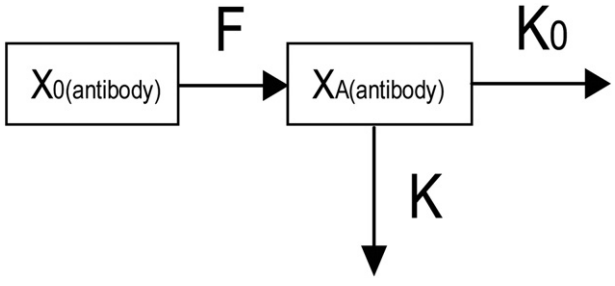

Fig. 1. Model diagram of the novel model used to describe the in vivo PK of mAbs in rabbits receiving an intravenous administration. As administrated intravenously, mAbs ($X_{0(antibody)}$) firstly go through an initial elimination by binding with the pre-existing targets, thus causing a decrease in its concentration (F). F can be estimated with the difference value between $X_{0(antibody)}$ and remaining mAbs ($X_{A(antibody)}$). Subsequently, $X_{A(antibody)}$ underwent a combined elimination of one first-order process (K) and another zero-order process (K_0).

The PK models were described by the following equations:

$$C = C_{A(antibody)}e^{-Kt} - \frac{K_0}{KV}\left(1 - e^{-Kt}\right) \quad (1)$$

Eq. (1) is applied to describe the PK process with an intravenous administration. $C_{A(antibody)}$ represents the maximum drug concentration in the plasma after drug is firstly administrated and occurs a quick binding with pre-existed targets; K represents the first-order elimination rate of mAbs, and it describes the kinetics of the pathway where mAbs eliminated through a phagocytosis of lymphocyte and/or a degradation and metabolism in liver; K_0 represents the zero-order elimination rate, describing the kinetics of the process where mAbs eliminate through binding with continuously generated targets, and in fact, it describes the zero-order generation rate of targets for the assumption that mAbs are dominant and newly generated targets are immediately captured; V is the volume of apparent distribution. Actually, parameters $C_{A(antibody)}$ and K_0 describe the kinetics of endogenous targets, and parameter K reflects the stability of mAbs in plasma.

2.2. Materials

Commercial immunoassay kits (Boster Biotechnology, China) were utilized to detect the plasma vascular endothelial growth factor (VEGF) concentration in rabbits. Ninety-six-well plates (Greiner, Germany) were used for an immunoassay of bevacizumab. Recombinant human $VEGF_{165}$ (Peprotech, USA) was immobilized on solid phase surface to capture bevacizumab. Bevacizumab (Avastin, 100 mg/4 ml) was obtained from the manufacturer (Genentech, CA, USA). Nonfat dried milk (Dingguo Changsheng Biotechnology, China) was selected as a sealed liquid, and phosphate-buffered saline (PBS) (Dingguo Changsheng Biotechnology, China) with an addition of 0.5% Tween-20 (Damao Chemical Reagent Factory, China) worked as a wash solution. Horseradish peroxidase-goat anti-human IgG (H + L) conjugate (ZSGB-BIO, China) was obtained to detect bevacizumab. Tetramethylbenzidine (TMB) (Solarbio, China) was purchased as a substrate solution, and 1 mol/L hydrogen chloride (Sinopharm Chemical Reagent, China) was prepared in the laboratory to work as TMB stop solution.

2.3. Pharmacokinetics in rabbits

The animal studies were approved by the Animal Ethics Committee of the Third Xiangya Hospital of Central South University. All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Twenty New Zealand rabbits with half males and half females, weighing 1.7 to 2.5 kg, were obtained from Slac Jingda Laboratory Animal Co., Ltd. (Changsha, China). All animals were provided with a 12 h light–dark cycle at an ambient temperature of 21–22 °C, and offered standard laboratory diet and water.

The rabbits were randomly divided into three groups among which no significant differences existed with respect to body weight and sex, and then received an intravenous infusion treatment of 15 mg/kg (group A), 5 mg/kg (group B), and 1 mg/kg (group C) of bevacizumab respectively. 1 ml blood was collected via ear vein before and 0.25, 1, 2, 4, 8, 12, 24, 48, 72, 120, 168, 216, 264, 336, 408, 480, 600, and 720 h after a single-dose administration. All plasma samples were separated following centrifugation at 3500 rpm for 10 min after collection, and then stored at −20 °C until analyzed.

2.4. Analytical method

An enzyme-linked immunosorbent assay was utilized to measure bevacizumab concentration as previously described with slight modification [27]. Firstly, Ninety-six-well plates were coated with