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REPORT

The role of Pharmacometrics in the development of Cuban Biotechnology products

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ABSTRACT

Pharmacometrics is a vibrant scientific discipline that involves a cycle of excellence: integration, innovation and impact. This work was aimed to assess different pharmacometric approaches in three Cuban biotechnological products. A population pharmacokinetic analysis of nimotuzumab was performed in patients with stage III breast cancer with different doses of it, in combination with doxorubicin and cyclophosphamide. The pharmacokinetic/pharmacodynamic (PK/PD) characterization of Pegylated Recombinant Human Erythropoietin (rHuEPO) branched 32 kDa-PEG-rHuEPO and 40 kDa- PEG-rHuEPO was conducted and compared with reference products (ior®EPOCIM and MIRCERA®) in New Zealand rabbits. Data were analyzed using the nonlinear mixed–effect approach (NONMEM®). The best model for nimotuzumab was the Quasi Steady State approximation of the full Target Mediated Drug Disposition model that best described the linear and nonlinear PK. The recommended optimal biological dose ranged between 200-400mg/week. On the other hand, a cell transit semi-mechanistic PK/PD model for characterizing rHuEPO profiles was obtained. The development of new branched PEG-chain formulations of rHuEPO improves its PK and PD properties, compared to those of commercially available formulations (*i.e.*, ior®EPOCIM and MIRCERA®). Due to its integrative nature and predictive value, population modeling was very useful in the optimal characterization of the pharmacokinetic and pharmacodynamic properties of these three Cuban biotech drug products. It had a significant impact on decision-making by both the national regulatory agency and the local biopharmaceutical industry as to their research and development plans, as well as the subsequent marketing strategies for these new products, with substantial economic and time saving benefits. This work received the Annual Award of the Cuban Academy of Sciences for the year 2019.

Keywords: Pharmacometrics, pharmacokinetics, pharmacodynamics, pegylated erythropoietin, nimotuzumab, NONMEM

RESUMEN

Aporte farmacométrico en el desarrollo de productos biotecnológicos cubanos. La farmacometría es una disciplina científica vibrante que está conformada por el ciclo de excelencia: integración, innovación e impacto. Objetivo: Evaluar diferentes enfoques farmacométricos en tres productos biotecnológicos cubanos. Se desarrolló un análisis farmacocinético poblacional del nimotuzumab en pacientes con cáncer de mama en estadio III, con escalado de dosis combinado con doxorrubicina y Cyclophosphamide. Se caracterizó la famarcocinética/farmacodinámica (PK/PD) de eritropoyetinas humanas recombinantes (EPOhr) pegiladas ramificadas (EPOhr-PEG2-32kDa y EPOhr-PEG2-40kDa), y se comparó con los productos de referencia ior®EPOCIM y MIRCERA® en conejos Nueva Zelanda. Para el análisis de los datos se empleó la modelación de efectos mixtos no lineales (NONMEM®). El mejor modelo obtenido para el nimotuzumab fue la aproximación cuasiestado estacionario del modelo completo de disposición mediado por el receptor. Este modelo permitió describir el comportamiento cinético lineal y no lineal del anticuerpo monoclonal. La dosis biológica óptima recomendada osciló entre 200 y 400 mg semanalmente. En el caso de las EPOhr se obtuvo un modelo semimecanístico PK/PD de tránsito celular. Las EPOhr-PEG2-32kDa y EPOhr- PEG2-40kDa mejoraron las propiedades PK y PD en comparación con ior®EPOCIM y MIRCERA®. La modelación con enfoque poblacional, por su carácter integrador y predictivo, fue de gran utilidad en la caracterización óptima de las propiedades PK y PD de los tres productos biotecnológicos cubanos. Además, tiene un notable impacto en la industria farmacéutica en

ahorro económico y en tiempo, así como, en el ámbito regulador, en la toma de decisiones durante el proceso de investigación, desarrollo y posterior comercialización de nuevos productos. Este trabajo mereció el Premio Anual de la Academia de Ciencias de Cuba para el año 2019.

Palabras clave: Farmacometría, farmacocinética, farmacodinámica, eritropoyetina pegilada, nimotuzumab, NONMEM

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Introduction

Pharmacometrics has become a vibrant scientific discipline in the world during the last decade, which is clearly expressed in the integration, innovation and impact triad. This triad forms a cycle of excellence in the discovery, development, research and approval of new drugs. It contributes, as a whole, in potentiating and hastening its development, expanding the evaluation spectrum, from basic research on the disease, to the mechanism of action of the drugs for their rational use in the care of patients [1].

Different studies in relevant species are required as a part of the development of a new pharmaceutical product. In these types of studies, pharmacometrics is used to develop pharmacokinetic, pharmacodynamic and pharmacokinetic/pharmacodynamic (PK/PD) models, among others. These pharmaco-statistic models make it possible to characterize, understand and predict the behavior of the PK and PD of a drug, quantify the uncertainty of the information on this drug and rationalize decision-making based on data produced during the development of the drugs and pharmacotherapy. The development and application of appropriate models are of great use in the regulatory field, since they notably contribute to relevant decision-making within the development of an R +D project for a new product, influencing the success of the approval process during its application for registration and later authorization for marketing [2, 3].

Therefore, in this work, the pharmacometric PK and PK/PD population approaches were applied for the first time in Cuba in the development of Cuban biotechnology products such as nimotuzumab and pegylated recombinant human erythropoietins (EPOhr) (EPOhr-PEG2-32kDa and EPOhr-PEG2-40kDa). The general aim of this study was to evaluate different pharmacometric approaches for Cuban biotechnological products, and their specific objectives. These objectives are the following: characterize the pharmacokinetic population of the combined schedule of nimotuzumab, Doxorubin and Cyclophosphamide in patients with locally advanced breast cancer, and to characterize the PK/PD population ratio of the pegylated branched erythropoietins of 32 and 40 kDa compared to the products of reference, i.e. ior®EPOCIM and MIRCERA®.

Materials and methods

Characterization of the nimotuzumab clinical trial

We designed a phase I, single-center, opened, uncontrolled, nonrandomized clinical trial (CT) with the scaling up of doses and multiple administrations of nimotuzumab, with the Cuban public registration number EC RPCEC00000057 that coincides with the WHO registration number [4]. This new therapeutic schedule for nimotuzumab included an increase in the number of administrations, mounting to ten. The first administration of this monoclonal antibody (MAb) was applied before the chemotherapy, so that the effect on the tumor expressing the HER1 receptor would start without the interference of the later effect of the cytostatic therapy. This would induce a maximum effect, and would also evaluate the PK of nimotuzumab in this type of patient, as well as the therapy. A sample of 12 female patients was selected according to the selection and exclusion criteria. Three patients were used at each dose level of 50, 100, 200 and 400 mg for the MAb, according to the designs for scaling-up doses using the modified Fibonacci method.

The study was conducted under the ethical principles of the Helsinki declaration, with the approval of the Ethics Committee for the protection of human subjects in the clinical trial at the Hermanos Ameijeiras Hospital (HAH) and at the Center for the State Control of the Quality of Drugs and Medical Devices (CECMED).

Populational pharmacokinetic analysis of nimotuzumab

The blood samples were collected immediately before the start, and after the end of the infusion on days 0, 7, 14, 21, 28, 35, 42, 49, 56 and 63 of the treatment (instances 1-10). The additional samples were obtained on days 1, 2, 4 and 6 after the first administration (instance 1) and days 64, 69, 77, 83 and 89 after the start of the treatment corresponding to 1, 6, 14, 20 and 27 days after the tenth administration (instance 10). The samples were incubated, centrifuged, and the supernatant (serum) was separated. It was then dispensed in aliquots of 50 µL and frozen at –20 °C. The serum concentrations of nimotuzumab were determined by a previously validated indirect ELISA method. We carried out the simultaneous analysis of all log-transformed serum concentrations vs. time, for the four doses assayed.

Characteristics of the PK/PD study of EPOhr formulations

The experimental formulations of EPOhr-PEG2-32kDa and EPOhr-PEG2-40kDa were developed by the Center for Molecular Immunology (CIM), Havana, Cuba. The commercial formulations ior®EPOCIM (nominal concentration of 10 000 IU/mL) and MIRCERA® (pre-loaded syringes of 100 µg/0.3 mL) were obtained from CIMAB S.A., and Hoffman-La Roche, respectively. The batches used in each one of

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the formulations under study were released according to the quality criteria established by CIM and following the USP XXII.

Each product was studied in New Zealand (NZ) male rabbits obtained at the National Center for the Production of Laboratory Animals (CENPALAB), which had their corresponding health and quality certificate. The rabbits were placed in individual cages under controlled conditions. The protocol was approved by the Animal Care and Use Committee of the Research and Biological Evaluations Center (CIEB, according to its Spanish acronym) of the Pharmacy and Food Institute of the University of Havana in compliance with the Directive for the Care of Animals of the European Union [5] and the ARRIVE guidelines for animal experimentation [6]. Eight experimental groups of five rabbits each were formed, for a total of forty animals. Four groups (I-IV) were used for the pharmacokinetics study, and the others (V-VIII) for the pharmacodynamics study. At the start of the study, the weight of the animals was within the range of 1.5 to 2.3 kg and age was of 24-28 weeks.

For the design of the PK and PD study, we started with equivalent concentrations of EPOhr. This was then administered through an intravenous bolus of 10 µg/kg of ior®EPOCIM, MIRCERA®, EPOhr- PEG2-32kDa and EPOhr-PEG2-40kDa in the left marginal vein of the ear of each animal of groups I and V, II and VI, III and VII, IV and VIII, respectively. In the PK study, the blood samples were collected at different time periods, i.e. 0, 0.5, 1, 3, 6, 8, 16, 24, 36, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h, except for the group with ior®EPOCIM, which were at 0, 0.5, 1, 3, 6, 8, 16, 24, 36 h. All serum samples were processed and quantified by the EPO ELISA commercial kit from ROCHE.

In the PD study for the groups treated with ior®EPOCIM and MIRCERA®, the blood samples were collected at 0, 3, 7, 10, 21, 36, and 42 days. For the groups treated with EPOhr-PEG2-32 and EPOhr-PEG2-40 they were collected at 0, 0.04, 0.125, 0.25, 0.33, 0.66, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 21, 36 and 42 days. Blood samples (1 mL) were placed in Eppendorf tubes containing 10 µL of EDTA (10 %) as the anticoagulant. Erythrocyte and hemoglobin determinations were made in a PENTRA 120 automatic hematologic analyzer. The reticulocytes were manually counted according to the clinical laboratory standards of the National Institute of Oncology and Radiobiology (INOR) and the reports from the literature [7].

Data were analyzed both for the characterization of PK of nimotuzumab and for the characterization of PK/PD of formulations of EPOhr with a population approach, through the NONMEM® software, version 7 [8] using the first-order conditional estimation method with the interaction option (FOCEI). We also used Xpose 4.0 [9], PsN 5.3 [10], R-package 3.0 [11] and Phoenix 6.3 [12] to guide the modeling and evaluation steps.

Results and discussion

Populational pharmacokinetic analysis of nimotuzumab

Up to 443 serum concentrations were analyzed, of which 84 belonged to instance 1; 288 to instances 2-9 and 71 to instance 10. The initial exploratory analysis of the data was carried out, and suggested a non-linear behavior in the doses tested of nimotuzumab (50-400 mg). The quasi-stationary state (QSS) approximation of the receptor target mediated drug disposition model (TMDD) remained as the best base model. This is a more mechanistic model, since it enables the study of the impact of MAb binding to its receptor on the distribution and elimination, i.e., on the disposition of the nimotuzumab. Furthermore, it also showed that the PK was found to be affected by the interaction of the MAb with its receptor, responsible for the biological response, because the kinetics is an auxiliary marker of the pharmacologic response. Figure 1 shows the base model that involved two compartments (central and peripheral compartments), the recycling of the receptor and the internalization/degradation of the MAb-receptor complex. The non-parametric bootstrap method with replacement was used to evaluate the stability of the final model and to construct the confidence intervals (CI) of the pharmacokinetic parameters.

After obtaining the base model, we started including a physiologically driven covariant approach with traits such as body weight, concentration of serum albumin, age, skin color, biochemical parameters indicating liver function, and these were evaluated with clearance (CL), and volume of central distribution (V_c). None of these covariates showed any statistically significant effect on the PK parameters. For this reason, the base model remained as the final model. The final parameters estimated by the PK population model and the bootstrap analysis with their confidence intervals are summarized in table 1. As shown, the standard errors of the parameters could not be estimated because of the unique algorithmic matrix. This is explained by the over-parameterization; given the complexity of the model, it was impossible to implement the covariance step to estimate the standard errors.

The volume of the population distribution for Vc and V_p was 1.43 L and 18.1 L, respectively. The total steady state distribution volume ($V_{SS} = V_C + V_P$) was 19.53 L, for an average body weight of 65 kg, suggesting an apparently limited distribution outside the vascular space in the extracellular volume. This value is high compared to that of cetuximab (V_{SS} = 5.26 L

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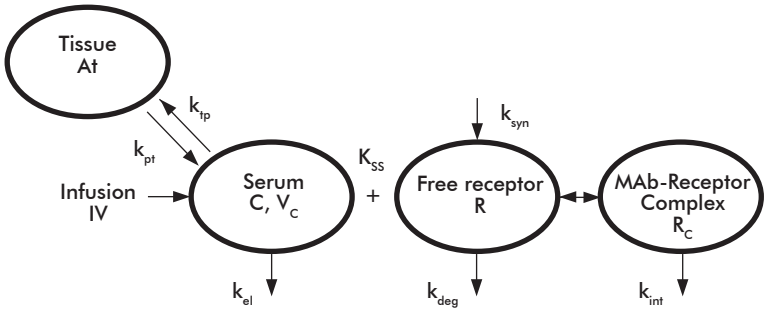

Figure 1. Diagram of the quasi-stationary state (QSS) approximation of the receptor-mediated disposition model. It includes the MAb-receptor link in the central compartment, recycling of the receptor, and the internalization of the MAb-receptor complex. At: amount of MAb in the tissue compartment. k_{pt}: first-order serum-tissue distribution rate constant. k_{tp}: first-order tissue-serum distribution rate constant. k_{el}: first-order MAb elimination rate constant. K_{SS}: concentration of the drug in which the interaction with the receptor is half the maximum value or constant at the steady state. k_{int}, k_{syn} and k_{deg}: constants for rates of internalization, production and degradation of the receptor, respectively.

for patients of 60 kg) [13] and matuzumab (V_{SS} = 5.57 L for patients of 71 kg) [14], which are approximately equal to the volume of the plasma, and consistent with the behavior of the endogenous IgG and of other therapeutic MAbs [15].

The K_{SS} (which is a value of concentration characterizing 50 % of linking the receptor in the steady state), was estimated with a value of 6.96 µg/mL, which was higher than the of the dissociation rate equilibrium constant (K_d) found in in vitro studies for nimotuzumab (1.5×10^{-4} µg/mL) [16]. The dissociation constant, K_d = koff/kon is a measure of affinity between the drug and the receptor in *in vitro* studies. However, the term $K_{SS} = K_d + (k_{int}/k_{on})$ is much more encompassing, since it includes the affinity constant and the complex internalization rate constant, and it can only be obtained *in vivo*. In this study, we estimated that the receptor has a fast recycling (K_{deg} = 5.50 h^{-1}, $t_{1/2}$ = 7.56 min of half-life) compared to the time scale of other processes such as that of the internalization of the MAb-receptor complex (K_{int} = 0.148 h^{-1}, $t_{1/2}$ = 4.68 h) and the half-life of nimotuzumab (483.71 h or 20.15 d). The latter was consistent with reports from the literature for IgG1, IgG2 and IgG4, which is of 21d [17-19].

The within-individual variability was associated to CL, V_C and K_{SS} with values of 11.31, 50 and 87.86 %, respectively. The within-instance variability [20] could not be included in CL. A residual error of 48 % was attained. The parameters of the final model generated from the bootstrap analysis were, in many cases, similar to the model developed for the 12 patients.

The percentage of the difference between the final model and the average data of the bootstrap analysis was below 17 % for all fixed parameters (structural pharmacokinetics) and for the random effects, with the exception of the CL (38.84 %), k_{deg} (33.27 %) and k_{int} (52.63 %) in which the prediction of the estimation was low. All the estimates of the final model were within the 95 % confidence interval obtained by the bootstrap method for which reason the QSS approximation of the TMDD was considered stable.

The total CL values of nimotuzumab (ranging from 4.5×10^{-3} L/h to 1.72×10^{-2} L/h or the equivalent range from 1.08×10^{-1} L/d to 4.13×10^{-1} L/d) were similar to the CL of the endogenous IgG, which is of 2.1×10^{-1} L/d [19]. The predictive capacity of the model was assessed through the predictive visual checking techniques corrected by the prediction and the posterior predictive check (PPC).

Through simulation, it was able to predict the concentrations of nimotuzumab after the weekly repeated dose regimen of 50, 100, 200, 400 and 1200 mg of nimotuzumab and a more extended time scale, corresponding to steady state instance 10, leading to a better appreciation of the non-linear behavior of this range of simulated doses. It was found that starting with the 200 mg dose, the receptor remained occupied (at 50 %) throughout the treatment. Mean values of AUC and the corresponding 95 % CI estimated from each simulation were 6624 (767.8-17 581) µg/mL•h^{-1}; 14121 (1685-35 949) µg/mL•h^{-1}; 30 685 (4017- 74732) µg/mL•h^{-1}; 75296 (11351-167519) µg/mL•h^{-1}; 316 633 (82 546-634 575) µg/mL•h^{-1} for the doses of 50, 100, 200, 400 and 1200 mg, respectively.

Table 1. Pharmacokinetic parameters estimated from the final PK population model and the bootstrap method

Parameters (units)	Estimates	Average results bootstrap (95 % CI)
PK parameters disposition		
CL (L/h)	7.03×10^{-4}	4.3×10^{-4} ($8{,}68 \times 10^{-5}$ – 1.39×10^{-3})
V_C(L)	1.43	1.38 (1.09 – 1.81)
VP (L)	18.5	21.47 (7.91 – 209.10)
CL_D (L/h)	3.22×10^{-3}	3.3×10^{-3} (2.1×10^{-3} – 5.1×10^{-3})
K_{SS} (µg/mL)	6.96	7.40 (1.04 – 150.40)
k_{int} (h^{-1})	1.48×10^{-1}	2.26×10^{-1} ($1.7x10^{-2}$ – 2.66)
k_{syn} (µg/mL)/h	1.43	1.46 (7.1×10^{-1} – 3.29)
k_{deg} (h^{-1})	5.50	7.33 (2.05 – 74.21)
$t_{1/2\beta}$ (h)	483.71	–
Between-patient variability*		
BPV_{CL} (%)	11.31	
BPV_{Vc} (%)	50.00	
BPV_{Kss} (%)	87.86	
Residual error (Additive on log-data; %)	48.00	
Shrinkage		
η-skrinkage (%)	9.55	
ε-*shrinkage (%)*	3.53	

* The standard errors of the parameters were not provided by the program because of the unique algorithmic matrix. BPV: Between-patient variability. RE: Residual error. BPV and RE are expressed as coefficients of variation (%). CL: clearance. V_c and V_p: volume of central and peripheral distribution, respectively. Vss: Volume of the steady-state distribution Vc +Vp = 19.93 L. CLD: distributional clearance . K_{SS}: concentration of the drug in which the interaction with the receptor is half the maximum value or constant at the steady state. k_{int}, k_{syn} and k_{deg}: constants for rates of internalization, production and degradation of the receptor, respectively. CI: confidence interval.

In the population pharmacokinetic characterization, the dose range proposed is 200-400 mg/week, as the optimum biological dose, because there is a decrease in the total clearance of the monoclonal antibody, indicating the saturation of the epidermal growth factor receptor. The tested methodology allows for the design of a dosing regimen for nimotuzumab for obtaining the optimum biological dose. It integrates all the pharmacokinetic and pharmacodynamic events constituting the mechanisms for distribution and elimination, and for statistically promoting the behavior that will occur in a larger number of subjects. This comprehensive result is a valuable contribution to the International Health Registration dossier and for the marketing of the product.

Analysis of the PK/PD model of EPOhr formulations

We included 266 measurements of EPOhr in the pharmacokinetic analysis of 19 rabbits. For the pharmacodynamic characterization there were used 799 observations of RET and RBC counts, and levels of HGB at each instance in 20 rabbits. The time course of the concentrations of EPO in the serum was characterized, and it was later described the time profiles of the three response readings of RET, RBC and HGB simultaneously. For this, it was used the method of population pharmacokinetic parameters [21] to link the PK profiles with the response model obtained.

Figure 2 shows the diagram of the final semi-mechanistic PK/PD cell transit model. The parameters related to the typical physiological system that can be estimated by the model are RET_0, RBC_0, F_{HGB}, γ, and mean transit time (MTT). The latter is interpreted as the number of transitions /ktr. The cell transit models represent a continuous maturation process from one compartment to the next that

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is ruled by MTT, which is modeled as a first order rate constant that is equal for all compartments [22]. This chain of compartments makes it possible to capture the delay between the effect of the drug on the stages of precursor cells and circulating cells [23].

The model incorporates two proliferations (P1 and P2, without available observations), a RET, a mature RBC, and an HGB compartment. The proliferation of P1 cells is represented by a first order rate constant, kprol. Afterwards, the cells mature sequentially through the different compartments (P2, RET and RBC) following the first order rate processes ruled by ktr. The elimination of RBC follows a first order rate process also represented by the kRBC parameter. There was considered a feedback mechanism triggered by the change of the RBC levels in relation to the initial conditions (RBC_0) and expressed as $(RBC_0/RBC)_{\gamma 1}$ affecting k_{prol}, where γ1 modulates the magnitude of the feedback mechanism. The fraction of HGB (FHGB) makes it possible to relate the continuous time profile of the HGB with the sum of cells of RET and RBC. The effects of the drug (EDRUG) are incorporated as stimulation on the rate of the maturation process of P2 to RET, as a linear function (SLP) of the predicted levels of EPO in the serum. The process for the disposition of the different formulations of EPOhr were better described with a two-compartment model (central and peripheral) and linear elimination, obtaining the respective pharmacokinetic parameters estimated by the population PK, as summarized in table 2. The differences in the disposition of the drug for each formulation are better described using different clearances and apparent volumes of distribution in the central compartment (V1), but with the same between-compartment clearance (Q) and the same apparent volume of distribution in the peripheral compartment (V2). Different structural models were constructed, assuming different parameters for each CL and/or V1, Q and/or V2 formulations, but none of them offered a significant reduction of the minimum value of the objective function, and for the goodness-of-fit graph compared to the final PK model selected. In this case, the inter-individual variability was included in CL and V1.

The different V1 were obtained for each formulation, where the highest values were for the derivatives of EPO-PEG, suggesting that the latter had greater internalization. This agrees with the work reported by other authors [24, 25]. The values of CL for EPOhr-PEG2-40 kDa, EPOhr-PEG2-32 kDa, ior®EPOCIM and MIRCERA® were of 4.34×10^{-3} L/h; 5.77×10^{-3} L/h; 1.02×10^{-1} L/h and 1.06×10^{-2} L/h, where significant differences were observed with much lower values for the pegylated derivatives. The inter-individual variability associated to CL and V1 were 50 and 60 %, respectively, with 20.5 % residual error.

All the parameters in the model were estimated with appropriate precision, as shown by the relative standard errors and the results of the bootstrap analysis with the confidence intervals, as shown in table 2. The PK/PD model integrates the parameters of the system (MTT, RET_0, RBC_0, F_{HGB}, γ) and those related to the derivatives of EPOhr (SLP, γ2 y γ3) as shown in table 3. The maturation of the hematopoietic cascade was described with the MTT parameter for all formulations, and because of convergence problems, this parameter was established according to the value published in rabbits, which was of 80 d [26]. The estimation of the recounts of basal cells for RET_0 (0.17 %) and RBC_0 (5.91 × 1012 L) in the model characterized the initial conditions of the erythroid cells before the administration of the dose. There were similar to reports by other authors [23, 27], obtaining an RBC0 value of 5.92×10^{12} L in a cell transit model. The HGB was predicted assuming a linear correlation of 40 %

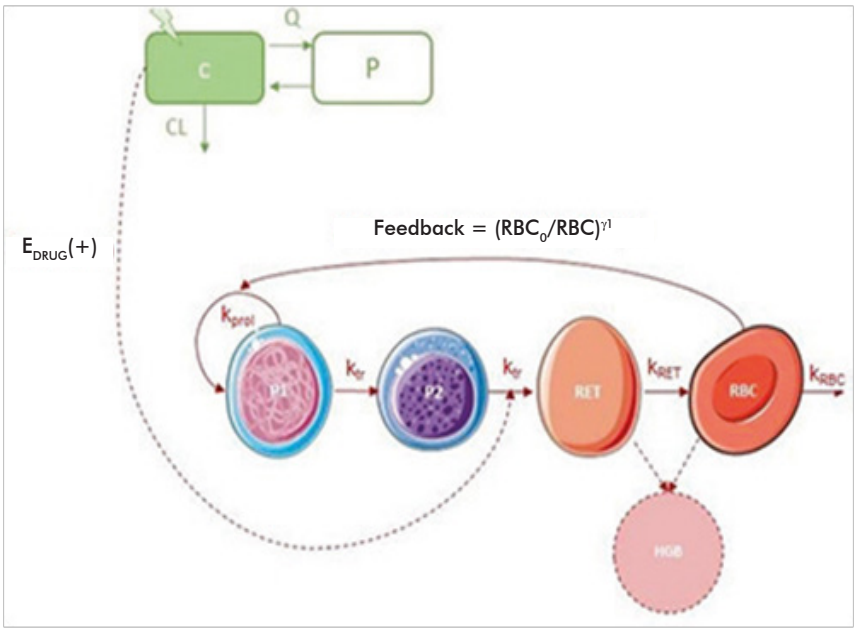

Figure 2. Diagram of the final semi-mechanistic PK/PD cell transit model corresponding to cellular proliferation and differentiation stages of erythropoietin. The disposition of EPO is described with a two-compartment model and linear elimination. P1 and P2 represent precursor compartments. The proliferation of P1 is represented by a first order rate constant, kprol. The cells mature sequentially through the different compartments, i.e., P2, RET and RBC, following first order rate processes k_{tr}, k_{RET} and k_{RBC}. $(RBC_0/RBC)_{\gamma 1}$ represents the feedback cycle considered, and E_{DRUG} corresponds to the effects of the drugs.

Table 2. Pharmacokinetic parameters estimated in the population of EPO formulations after the IV administration in NZ rabbits

Parameters (units)	Final PK model			Bootstrap analysis (n = 1000)		
	Average value	RSE (%)	Shrinkage (%)	Median	RSE (%)	CI 95 %
RE [CV (%)]	20.5	13	6	19.9	15	(14-26)
CL ior®EPOCIM (L/h)	1.02×10^{-1}	8	–	1.04×10^{-1}	9	$(0.5\text{-}1.5) \times 10^{-1}$
CL MIRCERA® (L/h)	1.06×10^{-2}	15	–	1.00×10^{-2}	14	$(0.6\text{-}1.6) \times 10^{-2}$
CL EPO-PEG-32 (L/h)	5.77×10^{-3}	26	–	$5.4\,0\times 10^{-3}$	31	$(3.5\text{-}9.8) \times 10^{-3}$
CL EPO-PEG-40 (L/h)	4.34×10^{-3}	28	–	4.30×10^{-3}	35	$(3.0\text{-}7.1) \times 10^{-3}$
V1 ior®EPOCIM (L)	1.94×10^{-1}	20	–	1.90×10^{-1}	22	$(1.1\text{-}2.9) \times 10^{-1}$
V1 MIRCERA® (L)	7.0×10^{-1}	12	–	6.70×10^{-1}	15	$(4.9\text{-}9.1) \times 10^{-1}$
V1 EPO-PEG-32 (L)	5.52×10^{-1}	29	–	5.40×10^{-1}	2	$(3.3\text{-}7.7) \times 10^{-1}$
V1 EPO-PEG-40 (L)	6.46×10^{-1}	34	–	6.40×10^{-1}	38	$(4.2\text{-}11.9) \times 10^{-1}$
Q (L/h)	2.24×10^{-1}	23	–	2.20×10^{-1}	29	(0.13-0.33)
V2 (L)	3.94×10^{-1}	16	–	3.90×10^{-1}	17	(0.28-0.45)
IIV CL [CV (%)]	50	18	2	49	19	(17-56)
CORR CL-V1 [CV (%)]	88.5	8	–	87	8	(65-97)
IIV V1 [CV (%)]	60	12	3	48	–	(23-65)

RE: residual error. IIV: variability between individuals. RSE: relative standard error. IC (CI): confidence interval; CV: coefficient of variation. CL: clearance. Vc and Vp: volume of central and peripheral distribution. Q: Distributive CL.

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(F_{HGB} = 0.4) with the sum of RET and RBC. This shows the interdependence of the three final pharmacologic points, and the possibility of deriving HGB in function of the observations of RET and RBC. The estimation of parameter γ1 gave a non-significant difference of 1 ($p > 0.01$). An initial decrease was observed in the values of RBC and HGB, which was probably associated to excessive sampling and blood loss within the first 24 h, and it was described with a zero order elimination of 1.90 × 10-2 IU/L/h of RBC with a duration of 24 h. Different amplification factors for ior®EPOCIM (γ2 = 3.11) and formulations of EPO-PEG (γ3 = 1.34) were introduced in the model to characterize the maximum levels of RET during the transduction process.

The erythropoietic effects at a single dose, through the IV route, of formulations derived from EPOhr were appropriately characterized on applying a linear effects model with a value of 1.6×10^{-3} L/IU for the commercial formulations (ior®EPOCIM y MIRCERA®) and 1.45×10^{-3} L/IU for branched pegylated formulations. This parameter indicates the relationship between the erythropoietic effect and the serum concentration of the different derivatives of EPOhr. Hence, this suggests that because the branched pegylated formulations have a higher value of SLP, they show a greater erythropoietic effect at a lower concentration value compared to ior®EPOCIM and MIRCERA®. In relation to the random effects, it was observed that the variability between individuals was associated to RET0, RBC0, TDUM and SLP, with values of 18, 5, 16 and 30 %, respectively. Furthermore, there were obtained two values of residual error, *i.e*., 23 % for RET and 5.7 % for RBC and HGB, since the determinations were carried out by different hematologic techniques.

Tables 2 and 3 show the results of the bootstrap (n = 1000) of the selected model. All parameters were estimated with a good precision, according to the values of EER and the bootstrap analysis. All estimates of the model were within the 95 % confidence interval, and therefore, the PK/PD cell transit model can be considered stable.

In order to evaluate the impact of different pegylated formulations in human beings, it was carried out a deterministic allometric scaling-up, using the PK population parameters in rabbits that were scaled-up according to body weight. We used the standard exponents of 0.75 for CL and 1 for the apparent distribution volume. The PK parameters in humans had a similar behavior to those of rabbits, but with higher values, since humans metabolize slower than rabbits. In both species we found a decrease of CL and an increase in the half-life duration of the EPO-PEG where MIRCERA® showed values of 115 h, which is consistent with Locatelli *et al*. for studies in healthy volunteers reporting t½ of 133 h [28]. In the case of branched EPOhr-32 and -40, they showed t½ of 166 and 300 h, respectively, which were equivalent to 7 and 13 days.

Table 3. Population pharmacodynamic estimations of EPO formulations after IV administration in NZ rabbits

Parameters (units)	Final PD model		Bootstrap analysis (n = 1000)		
	Average value	Shrinkage (%)	Median	RSE (%)	CI 95 %
RE RET [CV (%)]	23.3	5	23.9	13	(21.5-26.0)
RE RBC and HGB [CV (%)]	5.7	–	6.4	7	(5.7-7.0)
RET_0 (%)	0.17	–	1.73×10^{-1}	16	$(1.52\text{-}1.96) \times 10^{-1}$
RBC_0 (10^{12} cells/L)	5.91	–	6.00	11	(5.87 – 6.10)
MTT (d)	80	–	80	–	–
γ1	1	–	1	–	–
γ2	3.02	–	3.44	21	(2.11 – 3.99)
γ3	1.24	–	1.45	26	(0.91 – 2.25)
$SLP_{ior®EPOCIM/MIRCERA®}$ (L/UI)	1.06×10^{-3}	–	1.08×10^{-3}	36	$(0.76 – 1.54) \times 10^{-3}$
$SLP_{PEG\text{-}EPO\ 32\ and\ 40\ kDa}$ (L/UI)	1.45×10^{-3}	–	1.43×10^{-3}	11	$(1.14\text{-}1.87) \times 10^{-3}$
F_{HGB}	0.4	–	0.4	12	(0.39 – 0.41)
K_0 (UI/L/h)	1.90×10^{-2}	–	2.04×10^{-2}	8	$(0.58 – 3.10) \times 10^{-2}$
T_{DUM} (d)	1	–	1	–	–
IIV RET_0 [CV (%)]	18	14	19	19	(14-26)
IIV RBC_0 [CV (%)]	5	13	5	21	(2-7)
IIV T_{DUM} [CV (%)]	16	66	18	29	(11-29)
IIV SLP [CV (%)]	30	15	29	13	(13-58)

T_{DUM}: duration of the zero order process. RE: Residual error; IIV: inter-individuals variability; RSE: relative standard error; CI: confidence interval; CV: coefficient of variation.

Conclusions

This work evaluated different pharmacometric approaches, as the quasi-steady state approximation of the complete TMDD model for nimotuzumab and the semi-mechanistic PK/PD cell transit model for human recombinant erythropoietins. These models enabled the quantitative and mechanistic description of the pharmacokinetics and pharmacodynamics of these biotechnological products, and the proposal of the biologically optimum dose. The application of these models has a high impact, both in the pharmaceutical industry and in the regulatory field, since they help economize resources and time, as well as contributing to decision making during research, development and the later marketing of future drugs. This pharmacometric contribution in the characterization of Cuban biotechnological products creates a guideline for the development of this discipline in our country, starting at the academia and up to the regulatory framework.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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