Population pharmacokinetics of carvedilol enantiomers and their metabolites in type-2 diabetes and healthy subjects

Glauco H. B. Nardotto[1], Vera L. Lanchote[1], Eduardo B. Coelho[2], Oscar Della Pasqua[3,4]

[1] Faculdade de Ciências Farmacêuticas Ribeirão Preto, Universidade de São Paulo, Brasil.
[2] Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Brasil.
[3] Clinical Pharmacology Modelling & Simulation, GlaxoSmithKline, Uxbridge, United Kingdom.

[4]Clinical Pharmacology & Therapeutics Group, School of Life and Medical Sciences, University College London, United Kingdom.

*Correspondence to:

Prof Oscar Della Pasqua

Clinical Pharmacology & Therapeutics Group

University College London

BMA House, Tavistock Square

London, WC1H 9JP

United Kingdom

Email o.dellapasqua@ucl.ac.uk

Phone: +442078741544

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1. Introduction

Carvedilol is a β-adrenergic receptor antagonist, which does not appear to cause insulin resistance or worsen glycaemic control and lipids profile in patients with type-2 diabetes (T2DM), who are hypertensive or have cardiac heart failure (Fonseca et al., 2007; Bakris et al., 2004; Giugliano, 1997; Jacob et al., 1996). Conceptually, enantiomers often differ in terms of their pharmacokinetics and pharmacodynamics properties. In the case of carvedilol, stereoselective differences in pharmacokinetics occur not only to the primary moiety, i.e. the parent compound, but also to its metabolites (Nardotto et al., 2017). It is also known that the (S)-(-)-carvedilol is a non-selective β-adrenergic receptor antagonist, whereas both (S)-(-) and (R)-(+) isomers are approximately equipotent alpha-1 receptor antagonists (Tenero et al., 2000; Zhou and Wood, 1995; Neugebauer et al., 1990).

Even though β-adrenergic receptors antagonists are of great importance to control arterial pressure as well to reduce mortality due to coronary artery disease and congestive heart failure, these drugs tend to increase insulin resistance, enhancing the chance that a non-diabetic hypertensive patient also develops diabetes (Ayers et al., 2012; Bell et al., 2009; Torp-Pedersen et al., 2007; Dahlöf et al., 2005; Poole-Wilson et al., 2003; Gress et al., 2000). Moreover, in diabetic hypertensive patients, it has been shown that β-adrenergic receptors antagonists increase fasting glucose levels by 1.55 mmol/L and glycated haemoglobin (HbA1c) by 1%. They also produce increased total cholesterol and triglyceride levels, while reducing HDL (Bell et al., 2009; Bakris et al., 2004; Holzgreve et al., 2003; Dornhorst et al., 1985).

Given its favourable pharmacological profile, carvedilol represents the treatment of choice for patients with the aforementioned concurrent conditions. Nevertheless, carvedilol remains underutilised not only because of the reluctance to prescribe β-adrenergic antagonists to diabetes patients, but also because of the limited understanding of the potential pharmacokinetic and pharmacodynamic interactions between carvedilol and other antihyperglycaemic drugs. There is also very limited information about the impact of glycaemic control or lack thereof on hepatic metabolic capacity (Alvarez et al., 2015; Dostalek et al., 2012;). Therefore, it would be of great therapeutic interest to understand the implications of potential pharmacodynamic differences resulting from stereoselective metabolism.

Carvedilol is almost exclusively cleared as three main metabolites, namely 5'-hydroxyphenylcarvedilol (5OHC), 4'-hydroxyphenylcarvedilol (4OHC) and O-desmethylcarvedilol (DMC), all of which are further conjugated to glucuronide (Ohno et al., 2004; Neugebauer and Neubert, 1991; Möllendorff et al., 1987; Neugebauer et al., 1987). The CYP isozymes associated with the carvedilol enantiomers metabolism include CYP2D6, CYP2C9 and to a lesser extent CYP2E1, CYP1A2 and CYP3A4. The metabolites 5OHC and 4OHC are mainly formed by CYP2D6, whereas DMC formation is determined primarily by CYP2C9 (Oldham and Clarke, 1997). In addition to the multiple hepatic pathways, carvedilol disposition is also subjected to the effects of P-glycoprotein (P-gp), to which it binds as a substrate (Bart et al., 2005; Takara et al., 2004).

This study aims to investigate the implications of T2DM on the pharmacokinetics of carvedilol enantiomers using an integrated population pharmacokinetic modelling approach. Only patients with good glycaemic control receiving standard metformin

and glibenclamide doses were considered for the purposes of this analysis. It should be highlighted that glibenclamide is a substrate and competitive inhibitor of CYP2C9. Both glibenclamide and metformin are also competitive P-gp inhibitors (Holstein et al., 2012; Bessadok et al., 2011; Surendiran et al., 2011; Tirkkonen et al., 2010; Kim and Park, 2003; Golstein et al., 1999).

Previous data from this clinical study have shown that the pharmacokinetics of both enantiomers of the unchanged carvedilol is not affected by long-term co-administration of glibenclamide and metformin. This same study also revealed that the pharmacokinetics of carvedilol enantiomers is not altered in T2DM patients with good glycaemic control. By contrast, significant differences were observed in the exposure to OHC and DMC enantiomers (Nardotto et al., 2017). These findings provide the basis for further evaluation of the implications of drug-drug interaction and stereoselectivity in the pharmacokinetics of carvedilol. To our knowledge, this is the first time that such differences are characterised in a strictly quantitative manner, taking into account the contribution of different pathways and polymorphism in drug metabolism.

2. Methods

2.1. Study design

The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol and the informed consent were approved by the research ethical committees of the local Hospital and of the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil.

Details of the study design are provided in Nardotto et al. (2017). Briefly, 13 healthy

volunteers (9 male, 4 female) and 14 T2DM patients (9 male, 5 female) ranging in age 44-57 years were enrolled in the trial after having given their written informed consent. Eligible T2DM were required to have good glycaemic control (HbA1c < 7% and FPG ≤ 130 mg/dL) at study initiation and on treatment with glibenclamide (5 mg t.i.d.) and metformin (500 mg t.i.d.) for at least 3 months.

Clinical parameters measured or calculated for all subjects at the time of the study were within the normal range (see Table S1 in Supplement 1). No CYP2C9*3/*3 carriers were identified (Pan et al., 2016; Fernandes et al., 2011; Kirchheiner, 2003) and except for one patient, all subjects were classified as CYP2D6 extensive metabolisers (EM) using metoprolol as probe (Neves et al., 2016; Sohn et al., 1992). The exception was a female T2DM patient, who was classified as CYP2D6 poor metaboliser (PM). Furthermore, based on midazolam apparent clearance, all subjects exhibited *in vivo* CYP3A activity within the normal range (10-40 mL/min/kg) (Jabor et al., 2005; Lamba et al., 2002).

The study was performed according to a randomized crossover design, in which a single oral dose of racemic carvedilol (25 mg Carvedilat®, EMS Sigma Pharma, Hortolandia, SP, Brazil) or a single oral dose of racemic carvedilol, glibenclamide (5 mg, Daonil®, Sanofi-Aventis Farmacêutica Ltda, Suzano, SP, Brazil) and metformin (500 mg, Glifage®, Merk S.A., Rio de Janeiro, RJ, Brazil) were given to healthy volunteers (n=13) under fasting conditions with a washout period of at least 15 days between treatment occasions. Likewise, T2DM (n=14) also received the racemic carvedilol single oral dose (25 mg) simultaneously with the morning daily dose of metformin (500 mg/8h) and glibenclamide (5 mg/8 h). It should be highlighted that the used of different treatment conditions for both

antihyperglycaemic drugs in healthy subjects as compared to T2DM patients, i.e. chronic treatment vs. single dose was determined by clinical safety concerns.

Blood samples for PK analysis were collected at the following times in both occasions: pre-dose (before the carvedilol dose), then 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 15, 18 and 24 h after the carvedilol dose. Details of the stereoselective methods for the analsysis of the plasma concentrations of carvedilol and its metabolites can be found in Supplement 1.

2.2. Data sets

A meta-analytical approach was used to ensure accurate estimation of the parameters describing the disposition of carvedilol enantiomers after oral administration. The concentration *vs*. time profiles of carvedilol enantiomers after a single infusion of 12.5 mg racemic carvedilol (Neugebauer et al., 1990) was combined with the data obtained in the current study. Details of the literature data extraction and initial parameterisation can be found in Supplement 2. A flow diagram of the data analysis, including modelling building and validation steps, is shown in Figure 1. Given that individual pharmacokinetic data after a single infusion of racemic carvedilol were not available, parameter distributions were inferred indirectly from the overall dispersion of the data, as expressed by the standard deviation of volume of distribution and area under the plasma concentration *vs*. time curve (AUC). The digitised plasma concentration *vs*. time profile after intravenous administration were integrated to the oral data from the

current clinical trial. The combined data set was then analysed as described in the next paragraphs.

2.3. Population pharmacokinetic modelling

Modelling was performed using a non-linear mixed effects approach, as implemented in NONMEM v.7.2 (ICON Development Solutions, Ellicott City, MD, USA). Parameterisation was based on a multi-compartmental model, describing both carvedilol enantiomers and their metabolites. Model selection and parameter estimation was based on first-order conditional estimation method with interaction option (FOCE-I) (Bauer, 2011) (subroutine ADVAN6, TOL=3), using a GNU Fortran 4.6 compiler (Free Software Foundation, Inc.) and PsN v.3.5.3 (Perl-speaks-NONMEM, Uppsala University, Sweden) (Lindbom et al., 2005). R v.3.1.2 (R Development Group, Vienna) was used for data formatting, graphical and statistical summaries.

Model building criteria included: (i) successful minimisation, (ii) standard error of estimates, (iii) number of significant digits, (iv) termination of the covariance step, (v) correlation between model parameters and (vi) acceptable gradients at the last iteration (Duffull et al., 2004). Different structural models with first or zero order absorption, distribution and elimination, with or without absorption lag time were evaluated (Bellanti et al., 2016, 2014; Piana et al., 2014).

All fixed and random effects were introduced in a stepwise manner. Inter-individual variability in PK parameters was assumed to be log-normally distributed. Therefore, a parameter value of an individual (θ_i) is given by the following equation:

$$\theta_i = \theta_{TV} \times e^{\eta i}$$

where θ_{TV} is the typical value of the parameter in the population and η_i is assumed to be a random variable with zero mean and variance ω^2. The residual variability was described with a proportional error model. This means that for the j^{th} observed concentration of the i^{th} individual, the relation Y_{ij} applies:

$$Y_{ij} = F_{ij} + F_{ij} \times \varepsilon_{ij}$$

where F_{ij} is the predicted concentration and ε_{ij} the random variable with mean zero and variance σ^2.

2.3.1. Covariate selection

Covariate analysis was performed to explore measurable sources of variability in the model. The impact of body weight, BMI, age, gender, creatinine clearance, fasting glucose, glycated haemoglobin, and liver function parameters (ALT, AST, total bilirubin and albumin) were evaluated as continuous covariates on model parameters. In addition, disease condition (i.e., type-2 diabetes) and CYP2D6 phenotype (EM or PM) were treated as categorical variables and their effect parameterised as discrete changes to the reference population parameter values (see Supplement 3 for details).

A stepwise forward inclusion/backward elimination procedure was used for covariate selection. The covariates were introduced one by one and retained as significant if a decrease in objective function value (OFV) of at least 3.84 units ($p<0.05$) was observed. During the backward elimination procedure an increase in OFV of at least of 7.8 units ($p<0.005$) was used as threshold for statistical significance (Joerger, 2012; Wählby et al., 2002, 2001). Comparison of hierarchical models comprised goodness of fit (GOF), likelihood ratio test (Maitre et al., 1991)

and shrinkage values (Piana et al., 2014). GOF plots included population and individual predicted *vs.* observed plasma concentrations, conditional weighted residual *vs.* observed concentrations and time (Hooker et al., 2007).

2.3.2. Model diagnostics

Assessment of the predictive performance of the final model was based on graphical and statistical diagnostic criteria, including visual predictive checks (VPC), posterior predictive checks (PPC), bootstrapping, normalised predictive distribution errors (NPDEs) and mirror plots. Diagnostics were based on simulations of the pharmacokinetic profiles, with one thousand replicates per subject. Given the clinical relevance of total systemic exposure for the effects of carvedilol enantiomers and their metabolites, AUC estimates were used as metrics of performance for the posterior predictive checks. Simulated concentrations were integrated to calculate AUC values using the trapezoidal method (Yano et al., 2001). These simulations were complemented by bootstrapping procedures, which were aimed at identifying bias, stability and the accuracy of the parameter estimates (Dowd et al., 2015; Lindbom et al., 2005; Parke et al., 1999).

Finally, in order to assess general model performance in subsequent applications using simulations, normalised prediction distribution errors (NPDEs) were obtained based on the 'npde' package v. 2.0 in R (Comets et al., 2008). This was followed by an evaluation of the variance–covariance structure using mirror plots. Mirror plots can be generated by PsN and are implemented with the objective of exploring whether the random parameter distributions in a model reflect the data dispersion

observed at individual subject level in the original data set (Piana et al., 2014; Zhao et al., 2013).

3. Results

First order oral absorption and elimination processes without lag-time described the pharmacokinetics of carvedilol enantiomers and their metabolites. Plasma concentrations of carvedilol enantiomers and their metabolites following the administration of a single oral dose of 25 mg racemic carvedilol are presented in figure 2, as mean and 95% confidence intervals.

A diagram of the compartmental structure describing the absorption and disposition of the carvedilol enantiomers and their metabolites (OHC and DMC) is shown in figure 3. Different estimates were observed for the absorption rate constant, bioavailability, inter-compartmental clearance and volumes of distribution of each carvedilol enantiomer (tables 1 and 2).

Given that carvedilol is metabolised by CYP2D6 to both 4OHC and 5OHC, and by CYP2C9 to DMC, we have added the plasma concentrations of (R)-(+)-4OHC and (R)-(+)-5OHC as (R)-(+)-OHC in order to parameterise the clearance of (R)-(+)-carvedilol by CYP2D6. Likewise, the sum of the (S)-(-)-4OHC and (S)-(-)-5OHC was defined as (S)-(-)-OHC and used to estimate the clearance of (S)-(-)-carvedilol by CYP2D6. A similar approach was used for the plasma concentrations of each DMC enantiomer, which allowed the assessment of the clearance of (R)-(+)-carvedilol and (S)-(-)-carvedilol by CYP2C9. As the carvedilol enantiomers are not cleared only by CYP2D6 and CYP2C9, parameterisation of drug disposition needs to account for residual clearance by other pathways (residual clearance). This means that the total

clearances of carvedilol enantiomers are the sum of their clearances by CYP2D6, CYP2C9 as well as the residual clearance. Among all the demographic and baseline covariates, only T2DM and CYP2D6 phenotype were found to have a statistically significant effect on the clearances (CP2D6 and CYP2C9) of both carvedilol enantiomers. Carvedilol clearances by CYP2D6 and CYP2C9 were also found to be statistically significant different between the enantiomers (S)-(-) and (R)-(+). An overview of the final parameter estimates is shown in tables 1 and 2, respectively for the enantiomers (S)-(-) and (R)-(+).

Standard diagnostics measures, including goodness-of-fit (figure 4 and S6 (Supplement 3)) and visual predictive check (figures 5 and S7 (Supplement 3)) plots showed adequate performance of the model relative to the observed data. In addition, the NPDEs analysis revealed acceptable differences between model predictions and observations (see figures S8 to S10 in Supplement 3). Mirror plots indicate that the variance-covariance structure was well characterised, as the simulated datasets reproduced the dispersion pattern observed in the original data (see supplement 3 Figure S11 to S16). The last step in the evaluation of the performance of the final model included posterior predictive checks (PPC) based on AUC0-24. As shown in Figure 6 and figures S17 to S19 (Supplement 3), the model accurately predicted exposure to both the parent drug enantiomers and their metabolites.

4. Discussion

The current clinical trial was designed to evaluate the implications of stereoselective differences in the pharmacokinetics of carvedilol. Whereas we acknowledge that the sample size is relatively small to establish the relevance of

pharmacodynamic differences or the lack thereof on a wider patient population, it can be anticipated that the predicted and observed differences are unlikely to be clinically relevant.

The observed higher exposure to (R)-(+)-carvedilol is consistent with previous findings (Eisenberg et al., 1989; Furlong et al., 2012; Neugebauer et al., 1990). However, our analysis also reveals in a systematic manner is the interplay between stereoselective metabolic activity and polymorphism in isozymes. Despite the small sample size, inter-individual differences in drug metabolism were determined primarily by polymorphism in CYP2D6, which probably explains most of the variability in plasma concentrations of the metabolites, beyond the underlying effect of stereoselective disposition. Interestingly, the higher exposure to (R)-(-)-carvedilol appears to result from a small reduction in total clearance (33.10 *vs* 45.97 L/h, $p<0.05$) as well as to its higher bioavailability (25.40 % *vs* 16.43 %, $p<0.05$) and rather limited residual clearance (4.81 vs 28.15 L/h, $p<0.05$) (for further details see Tables 1 and 2). On the other hand, we found that for EM subjects (healthy volunteers or T2DM patients), CYP2D6 contributes significantly more to the clearance of (R)-(+)-carvedilol than (S)-(-)-carvedilol.

It can be hipothesized that the higher clearances by CYP2D6 for the both carvedilol enantiomers in T2DM patients as compared to healthy volunteers (7.28 vs 1.65 L/h for (S)-(-)-carvedilol and 13.70 vs 2.69 L/h for (R)-(+)-carvedilol) are probably due to a compensatory mechanism, i.e., as a consequence of the inhibition of CYP2C9 by glibenclamide (7.71 vs 16.17 L/h for (S)-(-)-carvedilol and 10.50 vs 25.60 L/h for (R)-(+)-carvedilol). Similarly, the lower clearance values of both carvedilol enantiomers by CYP2D6 in a single PM T2DM patient (0.49 vs 0.46 L/h respectively for (S)-(-) and

(R)-(+)-carvedilol) as compared to EM healthy subjects (1.65 vs 2.69 L/h respectively for (S)-(-) and (R)-(+)-carvedilol) seem to be compensated by a higher clearance by CYP2C9 (13.88 vs 24.35 L/h respectively for (S)-(-) and (R)-(+)-carvedilol in a PM T2DM patient and 16.17 vs 25.60 L/h respectively for (S)-(-) and (R)-(+)-carvedilol in EM healthy subjects), which cancels out the differences between the two groups.

As the lower plasma concentrations of (S)-(-)-carvedilol cannot be assigned solely to its lower bioavailability, but also to its higher systemic clearance, our results suggest that intestinal P-gp transport has a limited impact on stereoselective pharmacokinetics. In fact, previous reports have shown that glucuronidation of (S)-(-)-carvedilol is higher as compared to (R)-(+)-carvedilol (Hanioka et al., 2012; Ishida et al., 2008; Takekuma et al., 2007). This contrasts with the findings reported by Giessmann et al. (2004), who have attributed the lower plasma concentrations of S-(-)-carvedilol to the induction of intestinal P-gp by rifampin. Clearly, these authors have overlooked the fact that rifampin also induces CYP and UGT isoforms (Devineni et al., 2015; Kasichayanula et al., 2013; Wang et al., 2014; Williamson et al., 2013).

From a therapeutic perspective, the most important finding was to establish that the total clearances of (R)-(+)-carvedilol do not differ between EM healthy subjects or EM and PM T2DM. Likewise, the total clearance of (S)-(-)-carvedilol does not differ between the EM healthy subjects, EM and PM T2DM patients.

With regard to the potential impact of drug-drug interactions, metformin is unlikely to exert any influence on the pharmacokinetics of carvedilol as it is not a substrate, inducer or inhibitor of CYP isozymes (Manolopoulos et al., 2011; Maruthur et al.,

2014; Scheen, 2010). On the other hand, we cannot exclude the role of competitive antagonism between carvedilol and glibenclamide. This consideration arises from the predicted lower clearance by CYP2C9 in EM T2DM patients as compared to EM healthy subjects (7.71 vs 16.17 L/h for the (S)-(-)-carvedilol and 10.50 vs 25.60 for the (R)-(+)-carvedilol). We do not believe that such differences are a consequence of the underlying pathology (i.e.T2DM). Previous studies have shown that the clearance of tolbutamide, which is a CYP2C9 selective substrate, does not change in T2DM patients who show good glycaemic control (Dostalek et al., 2012; Ueda et al., 1963). We also highlight the fact that in the current trial the healthy subjects received only a single oral dose of glibenclamide (5 mg), which may not cause same level of CYP2C9 inhibition observed in T2DM patients after long-term treatment with the drug. This may explain why no significant covariate effect was observed for treatment (metformin and glibenclamide).

Our analysis has not identified any demographic covariate, such as body weight and other baseline characteristics. Such results could be anticipated, given the somewhat homogeneous group of patients and healthy subjects. However, this should not alter the conclusions drawn so far. A wider sample size, including some stratification of individuals with different metabolic phenotypes and body weight would have allowed for the identification of other potential covariates (e.g., co-medications) and higher precision for the parameter estimates.

2. Conclusion

In brief, the use of a meta-analytical approach for the characterisation of the stereoselective pharmacokinetics of carvedilol provided evidence of differences in the disposition of the (R)-(+)-isomer, which shows a lower total clearance than that of (S)-(-)-carvedilol. Interestingly, despite higher total clearance of the (S)-(-)-isomer, lower levels of (S)-(-)-carvedilol in plasma were explained by its lower bioavailability and higher clearance of alternative metabolic pathways (residual clearance), other than CYP. Most importantly, our analysis showed that T2DM does not affect the pharmacokinetics of carvedilol. Therefore, no dose adjustment for carvedilol is recommended for T2DM patients receiving glibenclamide and metformin.

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Supplementary material:

Supplementary data to this article can be found online at:

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Figure and Table Legends:

Figure 1: Flow diagram describing the model building process and meta-analytical approach used to combine published data on the pharmacokinetics of carvedilol after intravenous administration with the clinical trial data, in which racemic carvedilol was administered orally.

Figure 2: Plasma concentrations over time of the enantiomers of carvedilol, hidroxyphenylcarvedilol (OHC) and O-desmethylcarvedilol (DMC) following a single oral dose of 25 mg racemic carvedilol. Data reported as the mean and 95% confidence intervals.

Figure 3: Diagram of the PK model of carvedilol enantiomers and their metabolites OHC (hydroxyphenylcarvedilol) and DMC (desmethylcarvedilol). Ka: absorption constant rate; F: bioavailability and Q: inter-compartment clearance.

Figure 4: Goodness-of-fit (GOF) plots for the final pharmacokinetic model describing the absorption and disposition of carvedilol enantiomers. Observed carvedilol enantiomers concentrations (ng/mL) are plotted vs. population and individual predictions (top). Conditional weighted residuals (CWRES) are plotted against population predictions and time (bottom). The points represent the data. The solid gray line in each plot is the line of identity.

Figure 5: Visual predictive check of carvedilol enantiomers. Lines indicate the 5^{th}, 50^{th} and 95^{th} percentiles of observed plasma concentrations over time. Shaded area depict the 95% prediction intervals for the 5^{th}, 50^{th} and 95^{th} percentiles of the predicted plasma concentrations (n=1000 simulations).

Figure 6: Posterior predictive checks (PPC) of carvedilol enantiomers. Frequency histograms show the predicted distribution of simulated AUC_{0-24} values. The solid line depicts the geometric mean of the observed AUC_{0-24} in the clinical trial.

Table 1: Parameter estimates and bootstrap of fixed and random effects from the final (S)-(-)-carvedilol and its metabolites pharmacokinetic model.

Table 2: Parameter estimates and bootstrap of fixed and random effects from the final (R)-(+)-carvedilol and its metabolites pharmacokinetic model.