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Xenobiotica, 2011; 00(00): 000–000
© 2011 Informa UK, Ltd.
ISSN 0049-8254 print/ISSN 1366-5928 online
DOI: 10.3109/00498254.2011.604746

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RESEARCH ARTICLE

Pharmacokinetic and pharmacodynamic properties of carvedilol in fructose hypertensive rats

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Abstract

1.Cardiovascular effects and pharmacokinetics of carvedilol were assessed in fructose-fed rats using pharmacokinetic–pharmacodynamic (PK–PD) modeling.

2.Male Sprague–Dowley rats were randomly assigned to receive tap water (C rats) or fructose solution (10% w/v) (F rats) during 6 weeks. Effects of carvedilol (1–3 mg/kg i.v.) on blood pressure, heart rate and blood pressure variability were recorded. Carvedilol plasma pharmacokinetics was studied by traditional blood sampling. Relationship between carvedilol concentrations and their hypotensive and bradycardic effects was established by PK–PD modeling. Vascular sympatholytic activity of carvedilol was assessed by estimation of drug effects on low frequency blood pressure variability using spectral analysis.

3.A greater volume of distribution and clearance of S-carvedilol compared to R-enantiomer was found in both experimental groups. Although PK–PD properties of S-carvedilol chronotropic effect were not altered in F rats, hypertensive rats showed greater efficacy to the carvedilol hypotensive response after administration of the higher dose. A similar potency of carvedilol to inhibit sympathetic vascular activity was found in F rats.

4.Carvedilol showed enantioselective pharmacokinetic properties with increased distribution in F rats compared with normotensive animals. An enhanced hypotensive activity of carvedilol was found in F rats compared with C rats, which is not related to enhance sympatholytic activity.

Keywords: Carvedilol, enantioselective pharmacokinetics, hypertension, PK–PD modeling, sympathetic vascular activity, metabolic syndrome, fructose

Introduction

The metabolic syndrome is a clustering of metabolically related cardiovascular risk factors, including insulin resistance, abdominal obesity, elevated blood pressure, and lipid abnormalities (Alberti et al., 2006). Although the exact mechanism involved in the physiopathology of the metabolic syndrome is actually unknown, increased sympathetic drive seems to play a role in the development of several components of this pathology, such as visceral obesity, high blood pressure, and insulin resistance (Grassi et al., 2004; Grassi et al., 2006). In addition, several preclinical and clinical evidences have demonstrated that blood pressure variability (BPV) is an independent risk factor for the incidence of cardiovascular events associated to hypertension (Su et al., 2005; Höcht et al., 2010). Moreover, reduced heart rate variability and changes in blood pressure variation are nowadays accepted as contributors to cardiovascular disease in patients with metabolic syndrome (Pikkujämsä et al., 1998; Tentolouris et al., 2008). Therefore, treatment of hypertension associated to metabolic syndrome must not only reduce blood pressure levels but also their variability.

Beta blockers are an attractive therapeutic class for the antihypertensive therapy, considering their cardioprotective

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(Received 07 June 2011; revised 08 July 2011; accepted 08 July 2011)

effect. Nevertheless, most of beta blockers (e.g. atenolol) negatively affect insulin sensitivity, carbohydrate and lipid metabolism, and are therefore not recommended in metabolic syndrome (Carella et al., 2010). However, recent large studies have shown a better metabolic profile with newer third generation vasodilating beta blockers, including carvedilol and nebivolol, suggesting a possible therapeutic role of these beta blockers in hypertensive patients with metabolic syndrome (Carella et al., 2010).

Carvedilol is a racemic third generation beta blocker with both enantioselective pharmacokinetic and pharmacodynamic properties (Bartsch et al., 1990; Keating et al., 2006; Prakash et al., 2009). It also shows pleiotropic effects, including antioxidant activity, inhibition of apoptosis, anti-inflammatory action and mitochondrial protection (Ruffolo et al., 1990). Carvedilol enantiomers show different pharmacokinetic behaviour in normotensive animals, considering that the volume of distribution and clearance of S-carvedilol are greater with regard to the R-enantiomer (Fujimaki, 1992; Stahl et al., 1993). Carvedilol enantiomers also differ with respect to their affinity to β-adrenergic receptors. Only S-carvedilol blocks with high affinity both β_1- and β_2-adrenoceptors (Keating et al., 2006). Conversely, both R- and S-carvedilol show similar antagonistic properties on α_1-adrenergic receptors (Bartsch et al., 1990). Therefore, it is expected that carvedilol enantiomers contribute in a different manner to the chronotropic and the hypotensive response.

Although the pharmacokinetic and pharmacodynamic properties of carvedilol have been investigated in normotensive animals (Bartsch et al., 1990; Ruffolo et al., 1990; Fujimaki, 1992; Stahl et al., 1993), to the best our knowledge, studies regarding the impact of the hypertensive state in experimental models of metabolic syndrome on enantioselective pharmacological behaviour of carvedilol are lacking. The fructose-fed rat is an animal model commonly used to study the association between hypertension and metabolic disorders (Hwang et al., 1987; Catena et al., 2003; Hsieh, 2005). Fructose-fed rat mimics the hypertensive stage associated to the metabolic syndrome and develops an insulin resistance syndrome with a very similar metabolic profile to the human condition, including hyperinsulinemia, insulin resistance, hypertriglyceridemia, and decreased HDL cholesterol (Hwang et al., 1989; Tran et al., 2009).

Therefore, by using of enantioselective pharmacokinetic–pharmacodynamic (PK/PD) modeling, the aim of the present work was the extensive assessment of the in vivo cardiovascular properties of carvedilol racemics, including the effects on heart rate, blood pressure regulation and its action on short-term blood pressure variability.

Materials and methods

Animals and induction of hypertension

Male Sprague–Dawley rats were used (220–250 g). Animal experiments were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-3, revised 1985). Animals were maintained on a 12-h light/dark cycle. Rats were kept in a room at 22 ± 2°C and the air was adequately recycled. All animals were fed standard rodent diet (Asociación Cooperativas Argentinas, Buenos Aires, Argentina) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals, and 69% starch and vitamin supplements, containing the same amount of calories.

Rats were randomly divided into two groups: control ($n = 18$) with tap water to drink for 6 weeks and fructose treated ($n = 18$) with fructose solution (10% w/v) to drink for 6 weeks. Rats were weighed previously to dietary manipulation and at the end of study. At week 5, blood samples were collected from the retroocular plexus in fasting conditions (5 h), and centrifuged at 4°C. Plasma glucose and triglyceride levels were measured by means of spectrophotometry (Automatic Analyzer Abbott Spectrum CCX, Abbott diagnostics, Abbott Park, IL, USA) and commercial kits (Wiener Glycemia and TG Color GPO/PAP AA, enzymatic methods, Wiener Labs S.A.I.C, Rosario, Argentina).

Preparation of carvedilol formulation

Carvedilol (Droguerías Saporiti, Buenos Aires, Argentina; purity: 100.1%) is practically insoluble in water and therefore a special formula was prepared to allow intravenous administration of the drug at a dose of 1 and 3 mg/kg. The formula of carvedilol solution consisted of 0.1% or 0.3% (w/v) carvedilol, 0.5% (w/v) polyvinylpyrrolidone, 40% (v/v) propylene glycol, 10% (v/v) glycerine and purified water.

Experimental design

Rats were anaesthetized with ether and the left carotid artery and left femoral vein were cannulated with polyethylene cannulae containing heparinized saline solution (25 U/ml). Cannulae were tunneled under the skin and externalized at the back of the neck. Experiments were performed in freely moving animals 24 h after cannulae placement.

The day of the experiment, arterial cannulae was connected to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA) coupled to a Grass 79D polygraph (Grass Instrument, Quincy, MA). The polygraph was connected to a digital converter adaptor unit (Polyview, PVA 1, Grass-Astro Med, West Warwick, RI), and recordings were stored and analyzed with a software program (Polyview 2.3 Astro-Med). Baseline mean arterial pressure (MAP) and heart rate (HR) were estimated during an interval of 60 min. MAP was calculated as the sum of the diastolic pressure and one-third of the pulse pressure. HR was estimated tachographically by counting the pulsatile waves of arterial pressure recording.

Carvedilol, at a dose of 1 ($n = 6$ per group) and 3 mg/kg ($n = 6$ per group), or vehicle ($n = 6$ per group) were injected intravenously during 30 s in fructose and control rats. After carvedilol administration, MAP and HR were

continuously recorded and blood samples (100 μl) were collected from the arterial cannulae at the following time points: 5, 10, 15, 30, 60, 90, 120 and 180 min.

Analytical determination of carvedilol

Arterial blood samples (100 μl), collected in polypropylene microcentrifuge tubes containing 5 μl of heparinized solution, were centrifuged at 10,000 rpm for 10 min under controlled temperature (4°C). It is important to mention that blood sampling could alter pharmacokinetic and pharmacodynamic behaviour of antihypertensive drugs due to fluid loss. Nevertheless, in our experimental protocol we only extracted 800 μl of blood during 3 h period for estimation of plasma concentration of carvedilol. This volume is significantly lower than the recommended maximal volume of blood to be removed (3.5 ml) in a rat weighing 250 g (Aimone, 2005), and therefore it could be suggested that blood loss during our experimental protocol did not affect PK–PD properties of carvedilol.

Plasma supernatant (30 μl) was carefully separated and carvedilol was extracted by liquid procedure. Briefly, an aliquot of internal standard (2 μg/ml propranolol in methanol), 0.50 M sodium bicarbonate (50 μl) and dichloromethane (1 ml) were added to 30 μl of plasma sample. The mixture was vortexed for 2 min and centrifuged at 2000 rpm for 10 min. The organic layer was transferred into a conical tube and evaporated under nitrogen gas. The dry extract was reconstituted with 100 μl of mobile phase and injected into the chromatographic system.

Levels of R- and S-carvedilol in plasma samples were measured by normal phase liquid chromatography with fluorescence detection using a chiral column (Chirex (S)-ICA and (R)-NEA, Phenomenex) and a fluorescence detector (FL-3000, Thermo Finnigan, France) as described previously (Di Verniero et al., 2010). Briefly, the excitation and emission wavelengths used were 238 and 350 nm, respectively. Optimal composition of the mobile phase was achieved by a mixture of hexane: dichloromethane: ethanol: trifluoroacetic acid (65: 30: 5: 0.2). Retention time of R-carvedilol and S-carvedilol in our chromatographic conditions was 12.8 ± 0.3 min and 14.6 ± 0.4 min, respectively. Coefficient of variation of the chromatographic method was less than 5% and limit of quantification of R- and S-carvedilol was 20 ng.ml^{-1}. The intraday and interday coefficients of variation were 2.8% and 4.5%, respectively. The method was linear in the range of 20–1000 ng.ml^{-1} and samples with higher concentration of carvedilol were diluted with blank plasma in order to achieve concentrations within the validation range.

Estimation of blood pressure variability

Blood pressure variability was continuously estimated by determination of standart deviation and spectral analysis of 3 min periods of blood pressure recordings obtained from baseline and during regular times after carvedilol administration when the quality of the arterial blood pressure signal was visually considered to be satisfactory. According to previous work by other authors (Pladys et al., 2004), spectral analysis of the data was performed using the Fast Fourier Transform algorithm with a Hamming window (Polyview 2.3 Astro-Med). Spectral densities in the very low frequency range (VLF) (0.1–0.2 Hz), in the low frequency (LF) range (0.2–0.7 Hz), and in the high frequency range (HF) (0.7–2.5 Hz) were calculated (Pladys et al., 2004). Although LF variability is affected by sympathetic modulation of vascular tone, we used LF/HF ratio as an index of vascular sympathetic activity. The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF variability (Pladys et al., 2004; Souza et al., 2008).

Pharmacokinetic–pharmacodynamic analysis

Pharmacokinetics of total R- and S-carvedilol concentrations was estimated by applying a two-compartment, first-order elimination model. Non-linear least squares regression analysis was performed using the TOPFIT program (version 2.0, Dr. Karl Thomae Gmbh, Schering AG, Gödecke AG, Germany) that uses a cyclic three-stage optimization routine (one-dimensional direct search; vectorial direct search/Hooke–Jeeves modified; Gauss–Newton/Marquadt modified). Pharmacokinetic parameters were estimated using both micro and macroconstants. No weighing scheme was used during pharmacokinetic parameter estimation. The area under the curve (AUC) of carvedilol levels vs. time (from 0 to infinity) was calculated using the linear trapezoidal rule. AUC_{0-180} was assessed by subtracting C_{180}/β from$AUC_{0-\infty}$, where C_{180} is the carvedilol concentration at 180 min after drug administration and β the terminal elimination rate constant. Clearance (Cl) and steady state volume of distribution (Vd_{ss}) were calculated by standard methods (Gibaldi et al., 1982).

In the PK–PD relationship study of carvedilol, racemic carvedilol concentrations and S-carvedilol levels were related to blood pressure lowering and chronotropic response to carvedilol, respectively. Relative hypotensive and bradychardic response to carvedilol, expressed as percentage of reduction with regards to baseline values, was estimated at regular times by relating reduction in MAP and HR values to baseline MAP and HR during 30 min before drug administration.

In each experimental subject, pharmacokinetic and pharmacodynamic data were fitted simultaneously for estimation of carvedilol PK–PD parameters. As a time delay between carvedilol plasma concentrations and their cardiovascular effects was observed, a PK–PD model with a separated effect compartment was used for analysis of the data. In previous studies we have found a good correlation between the cardiovascular effects of carvedilol and their plasma levels by the application of PK–PD model with an effect compartment (Bertera et al., 2009; Di Verniero et al., 2010).

In each experimental subject, a non-linear regression of these data was carried out using the ADAPT II software package (D'Argenio et al., 1997) by means of the sigmoidal Emax equation:

$$Y = \frac{E_{max} * C_e(t)}{EC_{50} + C_e(t)}$$

where Y is the change in blood pressure or heart rate expressed as % of basal value, E_{max} is the maximal response, EC_{50} is the carvedilol concentration yielding half maximal response, γ the coefficient of Hill and $C_e(t)$ is the carvedilol concentration (S-carvedilol for the chronotropic response and RS-carvedilol for the hypotensive effect) in the effect compartment at t time. Unweight data were used during PK–PD analysis.

The following parameters of the PK–PD model were evaluated: EC_{50}, E_{max}, γ and $t_{1/2eq}$. The parameter $t_{1/2eq}$ is the equilibration half time between the plasma and the effect compartment and may be calculated from ln2/k_{e0}.

As reduction of vascular sympathetic activity of carvedilol is related to blockade of α_1-adrenoceptor, RS-carvedilol plasma concentrations were related to LF/HF ratio in order to establish PK–PD properties of the drug on sympathetic activity on the vascular system. In a previous work, we have found a good correlation between carvedilol plasma concentrations and the effect on LF/HF ratio by using a physiological indirect PK–PD model. Birefly, we assumed that the vascular sympathetic activity (LF/HF ratio) is produced constantly through a zero order kinetics (K_{in}) and removed in a first order kinetics with a rate constant K_{out} (Di Verniero et al., 2010). Carvedilol inhibits the production of the sympathetic tone (inhibition of K_{in}) thereby affecting its magnitude. In each experimental subject, effects of carvedilol on vascular sympathetic activity were related to drug levels in the central compartment by means of the following equation:

$$dR/dt = K_{in}\left[1 - \frac{C_c}{C_c + IC_{50}}\right] - K_{out}R$$

where dR/dt is the change in LF/HF ratio, C_c the racemic carvedilol concentration in the central compartment and IC_{50} is the drug concentration that produces 50% of vascular sympathetic tone inhibition. K_{out} was fixed as the function of K_{in} and the baseline response ($K_{out} = K_{in}/R_0$). PK–PD analysis of the data was carried out using the ADAPT II software package (D'Argenio et al., 1997). Unweight data were used during PK–PD analysis.

Table 1. Baseline metabolic and hemodynamic parameters in control and fructose rats.

Parameter	Control rats ($n=18$)	Fructose rats ($n=18$)
Glycemia (mg/ml)	1.36 ± 0.04	1.56 ± 0.03*
Triglyceridemia (mg/ml)	0.51 ± 0.07	1.02 ± 0.09*
MAP (mmHg)	105 ± 2	114 ± 2
HR (bpm)	382 ± 14	377 ± 12

*$p < 0.05$ vs. control rats.

Statistical analysis

Normal distribution of the data and the variables of the study were verified using the Kolmogorov–Smirnov test. Data were expressed as means ± SEM. Basal values of MAP, HR and LF/HF ratio were compared by means of Student's *t* test. Statistical analysis of carvedilol effects on MAP, HR and LF/HF ratio was performed by two-way analysis of variance (ANOVA) and the test of Bonferroni as *post hoc* test. Pharmacokinetic and PK–PD parameters were log transformed for statistical analysis in order to reduce heterogeneity of the variance and further compared by two-way ANOVA and the test of Bonferroni as *post hoc* test. Correlation between maximal plasma concentration (C_{max}):Dose ratio or AUC:Dose ratio and other pharmacokinetics parameters (Vd_{ss} and Cl) was studied by means of Pearson's test. Statistical tests were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, CA). Statistical significance was defined as $p < 0.05$.

Results

Basaline values of glycemia, triglyceridemia, MAP and HR in control and fructose rats are shown in Table 1. Compared with normotensive animals, fructose feeding increased glycemia, triglyceridemia and MAP without changing HR (Table 1). These results are in agreement with metabolic and hemodynamic profiles previously reported in this experimental model of metabolic syndrome (Hsieh, 2005; Mayer et al., 2007; Mayer et al.; 2008).

Carvedilol pharmacokinetics

Figure 1 shows the concentration-time profile of S-carvedilol and R-carvedilol plasma concentrations in control rats and fructose hypertensive rats after intravenous administration of 1 ($n=6$ for each group) and 3 mg/kg ($n=6$ for each group) of the drug. A biexponential decay of plasma carvedilol levels was found in all experimental groups compatible with a pharmacokinetic two-compartment model (Figure 1). The resulting pharmacokinetic parameters are shown in Table 2. No differences were found in constant of distribution and constant of elimination comparing all experimental groups.

A dose-dependent increase in the volume of distribution of both carvedilol enantiomers was found in normotensive control rats. Conversely, only volume of distribution of R-carvedilol showed dose dependency in fructose-fed rats. After administration of racemic carvedilol 3 mg/kg, the volume of distribution of S- and R-carvedilol was significantly reduced in fructose hypertensive animals compared to normotensive group. In addition, although clearance of both

enantiomers was not affected by the hypertensive stage induced by fructose feeding, S-carvedilol clearance showed a dose-dependent increase in control rats (Table 2). As a consequence of the dose dependence of the volume of distribution and clearance estimations, both maximal plasma concentration and AUC increased less than proportionally for both R- and S-Carvedilol in control rats and for R-isomer in fructose animals (Table 2). Confirming these results, C_{max} and AUC of both R- and S-carvedilol showed

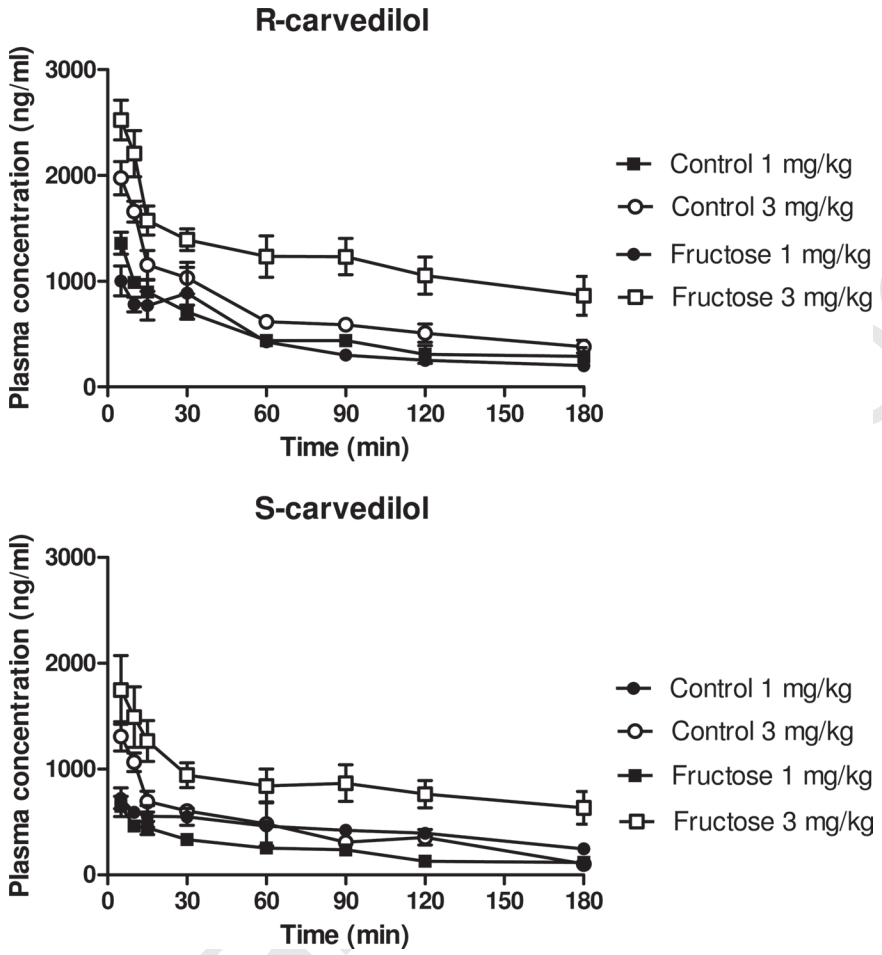

Figure 1. Mean plasma concentration values of S-carvedilol and R-carvedilol vs. time in control normotensive rats (circles) and fructose-fed animals (squares) after administration of 1 mg/kg (black symbols) and 3 mg/kg (open symbols) of the drug. Each point shows the mean ± SEM of six rats.

Table 2. Pharmacokinetic parameters of total S-carvedilol and R-carvedilol plasma levels obtained from arterial blood samples: AUC (area under the curve), α (constant of distribution), β (constant of elimination), Cl (clearance) and Vdss (steady state volume of distribution), Cmax (extrapolated maximal concentration) in control rats and fructose treated animals after i.v. administration of drug (1 mg/kg and 3 mg/kg).

Enantiomer	S-carvedilol				R-carvedilol			
Experimental group	Control rats		Fructose rats		Control rats		Fructose rats	
Dose	1 mg/kg(n=6)	3 mg/kg(n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)
α (h^{-1})	12.3 ± 1.2	8.0 ± 1.3	14.3 ± 1.4	9.3 ± 1.9	7.8 ± 1.8	6.3 ± 1.3	8.3 ± 1.2	8.0 ± 1.5
β (h^{-1})	0.43 ± 0.15	0.57 ± 0.07§	0.44 ± 0.09	0.38 ± 0.09	0.38 ± 0.19	0.25 ± 0.04	0.47 ± 0.07	0.31 ± 0.08
Vd_{ss} (l)	1.13 ± 0.26	2.24 ± 0.29$^{\#\S}$	1.14 ± 0.11§	1.21 ± 0.07§*	0.77 ± 0.11	1.51 ± 0.09$^{\#}$	0.53 ± 0.03	0.89 ± 0.07$^{*\#}$
Cl ($ml.min^{-1}$)	6.5 ± 1.4	14.2 ± 3.4$^{\#\S}$	9.1 ± 1.0§	8.9 ± 1.6§	4.5 ± 1.2	6.7 ± 1.1	4.4 ± 0.5	4.7 ± 1.3
C_{max} ($\mu g.ml^{-1}$)	1.61 ± 0.09	2.49 ± 0.34	1.64 ± 0.15	3.30 ± 0.26	1.60 ± 0.18	2.77 ± 0.32	2.02 ± 0.32	3.50 ± 0.22
$AUC_{0-\infty}$ (ng. $ml.h^{-1}$)	1675 ± 372	2487 ± 665	1000 ± 141	3802 ± 766*	2447 ± 487	4300 ± 803	2064 ± 296	4273 ± 609*

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).
$^{\#}p < 0.05$ vs. 1 $mg.kg^{-1}$.
$^{*}p < 0.05$ vs. Control rats.
$^{\S}p < 0.05$ vs. R-carvedilol.

a significant negative correlation with Vd_{ss} and Cl, respectively (Figures 2 and 3).

PK–PD modeling of the carvedilol chronotropic effect

Figure 4 shows HR changes time profile in control and fructose rats after vehicle or carvedilol intravenous administration at a dose of 1 and 3 mg/kg. Vehicle administration ($n = 6$ for each group) did not modify HR in either experimental group (Figure 4). The chronotropic response to carvedilol was not significantly different comparing Fructose feeding rats (–20.4 ± 3.2% for 1 mg/kg, $n = 6$;–30.4 ± 3.7% for 3 mg/kg, $n = 6$) with Control animals (–18.9 ± 2.3% for 1 mg/kg, $n = 6$;–24.8 ± 1.5% for 3 mg/kg, $n = 6$) after administration of both doses.

When correlating the chronotropic response to S-carvedilol concentrations, an effect compartment PK–PD model with sigmoidal E_{max} equation fitted well in all experimental groups (Table 3). No differences were found in E_{max} estimation comparing both dose levels in control and fructose animals (Table 3), suggesting that the complete pharmacodynamic range of carvedilol bradycardic effect was attained under our experimental conditions.The rate of carvedilol distribution at the biophase did not differ when comparing all experimental groups (Table 3). In addition, maximal chronotropic response and potency of S-carvedilol were similar comparing fructose-fed rats and normotensive control rats.

PK–PD modeling of the carvedilol hypotensive effect

Figure 5 shows the MAP changes time profile in control and fructose-fed animals after vehicle or carvedilol intravenous administration at a dose of 1 and 3 mg/kg. Vehicle administration (= 6 for each group) did not modify blood pressure in either experimental group (Figure 5). The hypotensive response to carvedilol was significantly greater in fructose feeded rats (–30.3 ± 2.6%, $n = 6$, $p < 0.05$) compared with control rats (–19.6 ± 1.7%, $n = 6$) after intravenous administration of carvedilol 3 mg/kg.

When correlating the blood pressure lowering response to racemic carvedilol concentrations, the effect compartment PK–PD model with sigmoidal Emax equation fitted well in all experimental groups. No differences were found in E_{max} estimation comparing both dose levels in control and fructose hypertensive rats (Table 4),

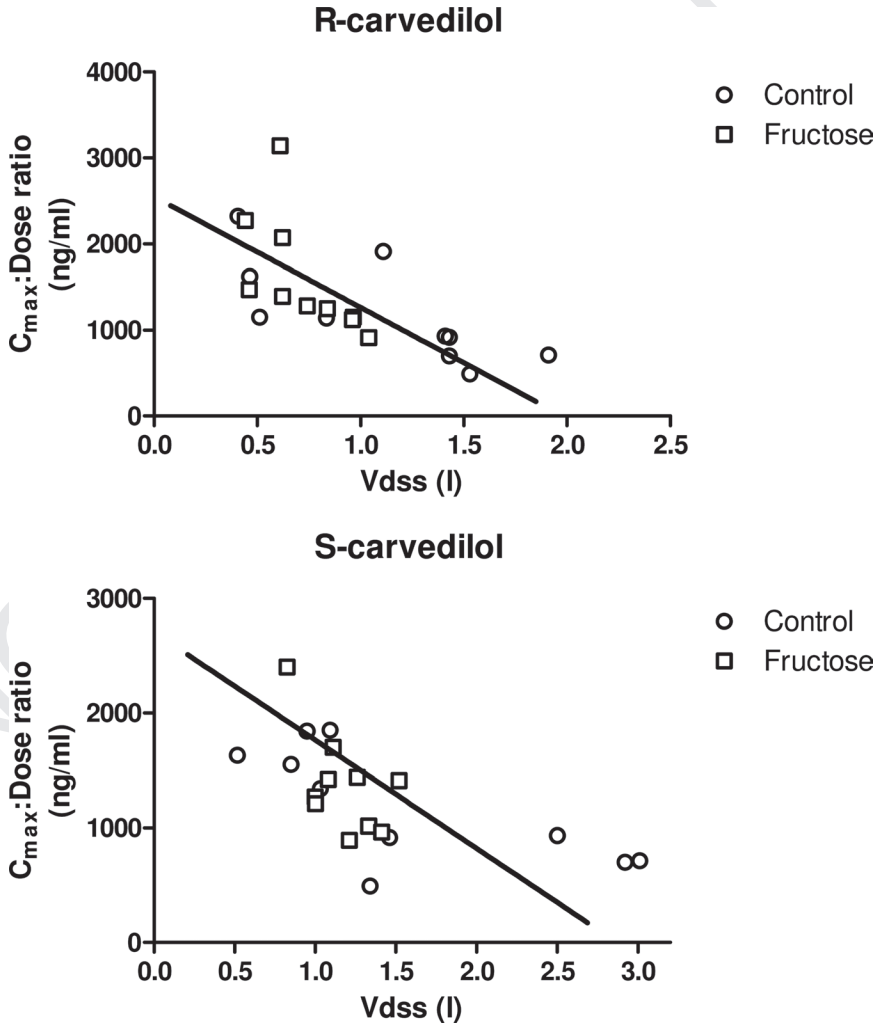

Figure 2. Correlation between maximal plasma concentrarion (C_{max}):Dose ratio of R-carvedilol and S-carvedilol and steady state volume of distribution (Vdss) in control and fructose rats. A significant negative correlation was found between Cmax:Dose ratio and Vd_{ss} for both R-carvedilol ($r = –0.5943$) and S-carvedilol ($r = –0.6280$).

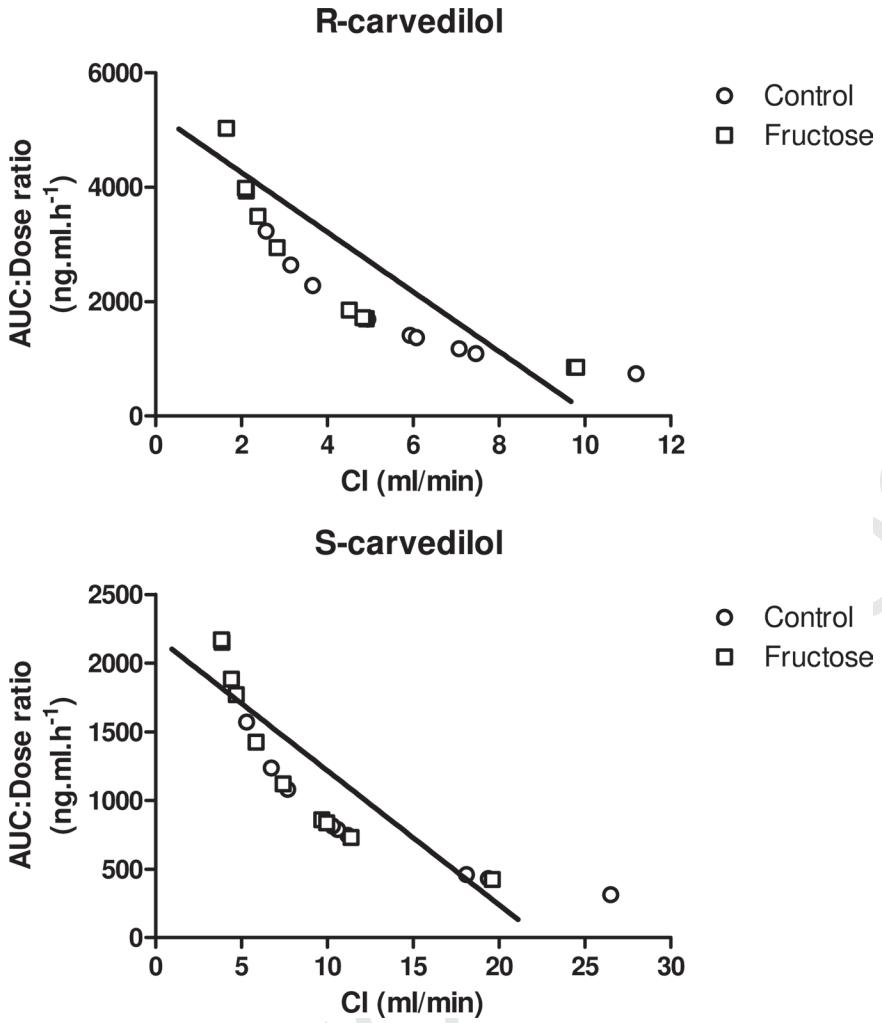

Figure 3. Correlation between area under the curve (AUC):Dose ratio of R-carvedilol and S-carvedilol and clearance (Cl) in control and fructose rats. A significant negative correlation was found between AUC:Dose ratio and Cl for both R-carvedilol ($r = -0.8708$) and S-carvedilol ($r = -0.8520$).

suggesting that the complete pharmacodynamic range of the carvedilol hypotensive effect was attained under our experimental conditions and the sigmoidal Emax equation is suitable for PK–PD parameter estimation.

Rate transfer of carvedilol from the central to the effect compartment did not differ in all experimental groups (Table 4). The maximal hypotensive response was significantly greater in fructose-fed rats compared with normotensive control rats. Potency of carvedilol hypotensive response was similar comparing both experimental groups with a non-significant increase of EC_{50} with dose increment (Table 4).

Effect of carvedilol on blood pressure variability and vascular adrenergic tone

Fructose hypertensive rats showed increased blood pressure variability compared with control rats. While both VLF and LF variability was greater in Fructose rats (VLF 19.0 ± 1.9 mmHg2; LF 12.8 ± 1.3 mmHg2; $n = 18$, $p < 0.05$) compared with the normotensive group (VLF 12.7 ± 1.0 mmHg2; LF 9.1 ± 0.5 mmHg2; $n = 18$) (Figures 6 and 7), no difference was found in HF variability between experimental groups (Control rats: 3.2 ± 0.3 $mmHg^2$, $n = 18$; Fructose: 4.1 ± 0.5 $mmHg^2$, $n = 18$). Vascular sympathetic activity estimated by baseline LF/HF ratio was not significantly different in fructose hypertensive rats (3.5 ± 0.2, $n = 18$) compared to normotensive control animals (3.2 ± 0.2, $n = 18$).

On the other hand, carvedilol administration significantly reduced BPV in the VLF and LF domain in control and fructose rats (Figures 6 and 7) without affecting HF variability of blood pressure in both experimental groups (data not shown). Vehicle administration ($n = 6$ for each group) did not modify blood pressure variability in fructose and control rats (data not shown).

Figure 8 shows the LF/HF ratio—time profile after vehicle or carvedilol (1 and 3 mg/kg) administration in control ($n = 6$ for each dose level) and fructose-fed rats ($n = 6$ for each dose level). While vehicle administration did not modify the LF/HF ratio in both experimental groups (Figure 8), carvedilol administration induced a similar reduction of this parameter in normotensive control rats and hypertensive frutose animals (Figure 8).

When correlating sympathetic vascular activity expressed as LF/HF ratio to racemic carvedilol plasma concentrations, the inhibitory physiological indirect PK–PD model fitted well in all experimental groups (Table 5). No differences were found in K_{in} and IC_{50} estimation comparing control and fructose rats. Estimation of PK–PD parameters for the carvedilol effect on sympathetic vascular tone did not change with dose increment in either experimental group.

Discussion

This study yielded several findings regarding enantioselective PK–PD properties of carvedilol in fructose hypertensive rats. Carvedilol enantiomers show different pharmacokinetic behaviour, considering that clearance and volume of distribution of S-carvedilol are significantly greater than R-carvedilol. Both enantiomers exhibit non-linear pharmacokinetics and the volume

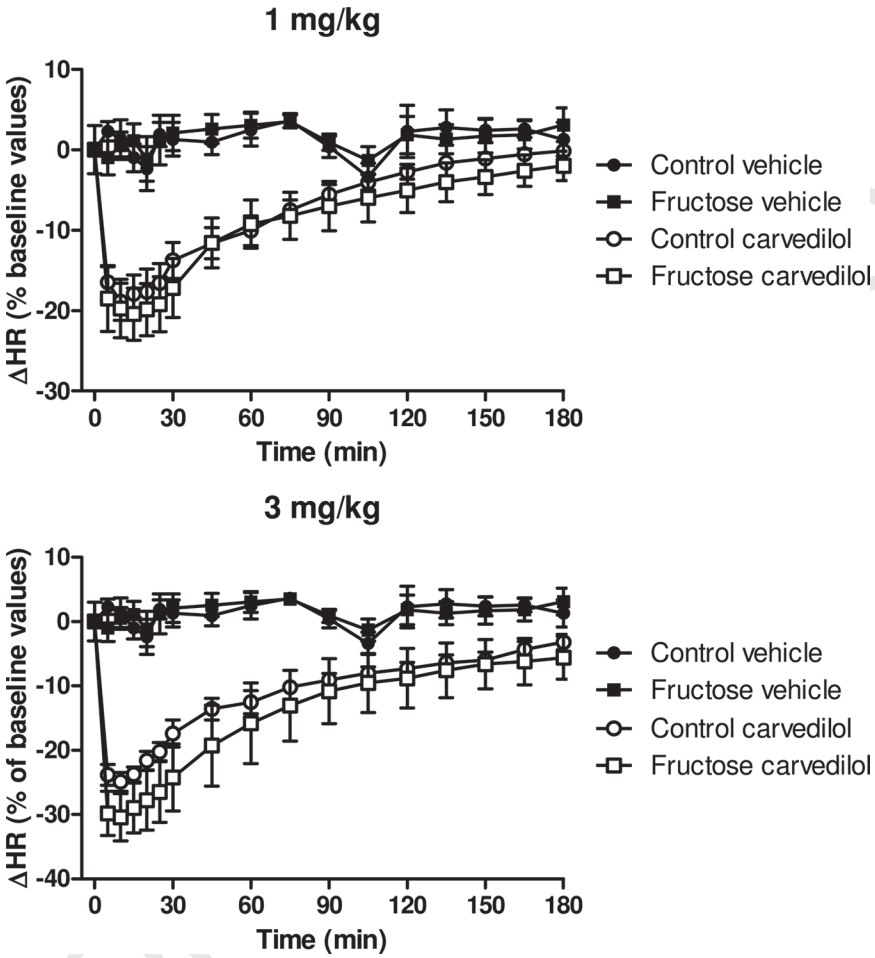

Figure 4. Time course of changes in heart rate (ΔHR, % of baseline values), after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats. *$p < 0.05$ vs. control rats.

Table 3. Resulting pharmacokinetic–pharmacodynamic parameters from the chronotropic effect of carvedilol in control rats and fructose-fed treated animals after i.v. administration of drug (1 and 3 mg/kg): EC_{50}: concentration yielding half maximal response, E_{max}: maximal response, γ: coefficient of Hill, $t_{1/2eq}$: equilibration half-life between the plasma and the effect compartment.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg(n=6)	3 mg/kg (n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)
E_{max} (%)	20.5 ± 2.5	26.5 ± 2.5	22.4 ± 3.4	31.1 ± 3.6
EC_{50}(μg/ml)	0.63 ± 0.11	0.67 ± 0.15	0.72 ± 0.09	0.84 ± 0.15
γ	2.2 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.4 ± 0.2
$t_{1/2eq}$ (min)	5.2 ± 1.5	3.2 ± 1.0	4.5 ± 1.2	2.1 ± 0.8
r^2	0.957 (0.922–0.982)	0.938 (0.881–0.985)	0.905 (0.839–0.987)	0.945 (0.875–0.978)
AIC	62.9 (52.8–76.7)	74.9 (62.5–89.7)	76.6 (55.8-125.8)	68.5 (47.1–93.4)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).

of distribution of of S- and R-carvedilol is reduced in hypertensive fructose rats compared with control rats after administration of the higher dose. The hypotensive response to carvedilol is enhanced in fructose-fed animals with regard to control normotensive rats, although reduction of heart rate and vascular sympathetic activity were similar comparing both experimental groups. These results suggest that other mechanisms involved in the antihypertensive response of carvedilol, such as its antioxidant activity, are enhanced in fructose rats with regards to control animals.

Carvedilol pharmacokinetics have been studied previously in both human volunteers (Neugebauer et al., 1990; Zhou et al., 1995; Phuong et al., 2004) and rats (Fujimaki, 1992; Stahl et al., 1993; Di Verniero et al., 2010). Carvedilol enantiomers show high plasma protein binding and metabolize through hepatic cytochrome P450 2D6 and P450 1A2, and intestinal cytochrome P450 3A4

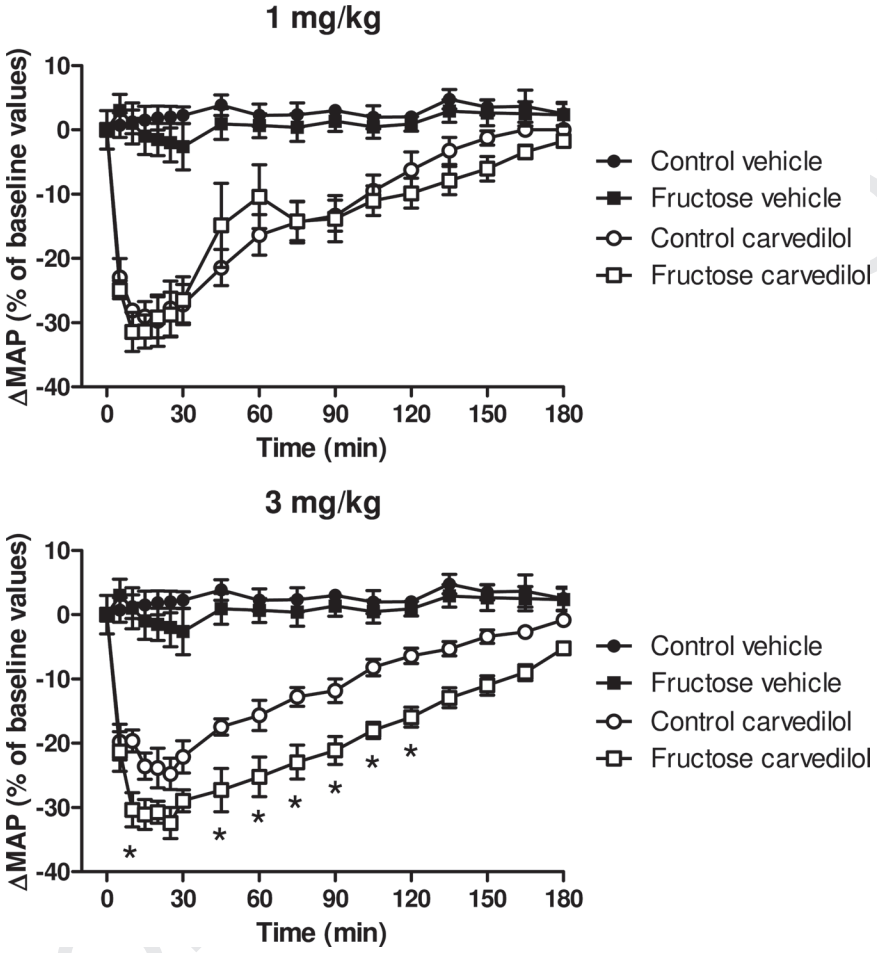

Figure 5. Time course of changes in mean arterial pressure (ΔMAP, % of baseline values), after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats.

Table 4. Resulting pharmacokinetic–pharmacodynamic parameters from the hypotensive effect of carvedilol in control rats and fructose-fed animals after i.v. administration of drug (1 and 3 mg.kg^{-1}): EC_{50}: concentration yielding half maximal response, E_{max}: maximal response, γ: coefficient of Hill, $t_{1/2eq}$: equilibration half-life between the plasma and the effect compartment.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg(n=6)	3 mg/kg(n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)
E_{max} (%)	30.6 ± 1.5	24.5 ± 2.1	34.7 ± 3.9	35.0 ± 2.8*
EC_{50}(μg/ml)	0.63 ± 0.11	0.68 ± 0.52	0.72 ± 0.09	0.83 ± 0.14
γ	2.2 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.4 ± 0.2
$t_{1/2eq}$ (min)	5.5 ± 1.2	4.2 ± 1.4	5.7 ± 1.8	7.6 ± 1.4
r^2	0.955 (0.922–0.982)	0.956 (0.881–0.985)	0.949 (0.839–0.987)	0.985 (0.875–0.978)
AIC	61.5 (51.5–73.2)	64.2 (60.5–85.7)	66.6 (58.8–105.6)	62.5 (47.1–92.6)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).
*p < 0.05 vs Control rats.

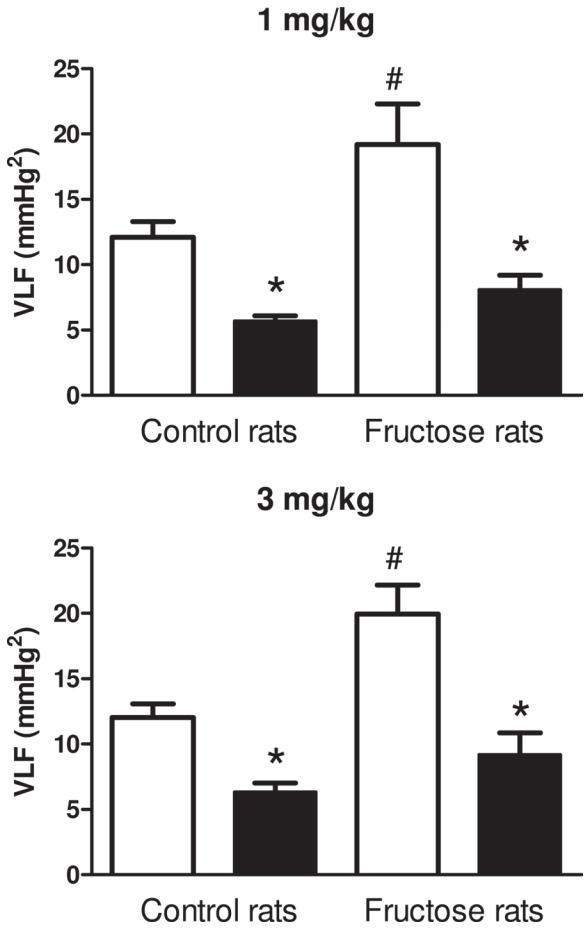

Figure 6. Mean very low frequency (VLF) variability of blood pressure in control and fructose-fed rats at baseline and after 30 min of carvedilol administration. Each bar shows the mean ± SEM of six rats.

(Ishida et al., 2008). The extraction fraction of carvedilol is high, showing an oral bioavailability of 0.19 and 0.83 in human volunteers and patients with cirrhosis, respectively (Verbeeck, 2008). In addition, several studies have described an enantioselective pharmacokinetic profile of carvedilol enantiomers: S-carvedilol shows a greater volume of distribution, clearance and presystemic elimination with regard to R-carvedilol (Fujimaki, 1992; Stahl et al., 1993; Di Verniero et al., 2010). In agreement with these findings, we found higher values for Vd_{ss} and Cl of S-carvedilol compared with R-carvedilol in both normotensive control rats and hypertensive fructose treated rats.

We studied carvedilol pharmacokinetics 24 h after arterial cannulation in rats. It has been demonstrated that surgical implantation of cannulae 24 h before measurements are taken induced an increment of α_1-glycoprotein (Terao et al., 1983). Although α_1-glycoprotein binds basic drugs, carvedilol binds predominantly to serum albumin (Stahl et al., 1993; Frishman, 1998) and, therefore, it seems unlikely that an increase in α_1-glycoprotein due to cannulae implantation would affect the carvedilol free fraction in our experimental conditions.

The relationship between carvedilol pharmacokinetics and dosing was assessed after administration of 1 and 3 mg/kg of the drug. Linear pharmacokinetics of carvedilol has been described in elderly subjects after oral administration of 25–50 mg of the drug (Louis et al., 1987). Conversely, a saturable first-pass effect for carvedilol was found in rats after high oral racemate dosing (Stahl et al., 1993). Our results suggested that, after application of a single intravenous dose over the range of 1–3 mg/kg, both S- and R-carvedilol showed a non-linear pharmacokinetic pattern in control and, only R-carvedilol, in fructose-fed rats mainly as a consequence of an increased Vd_{ss}. In addition, while both S- and R-carvedilol clearance did not change with dosing in fructose rats, S-carvedilol clearance showed a dose-dependent enhancement in control normotensive rats. A similar non-linear profile of carvedilol pharmacokinetics was previously found in Wistar normotensive rats and N^G-nitro-L-arginine methyl ester (L-NAME) hypertensive animals (Di Verniero et al., 2010). Considering that the lower than expected increase of C_{max} and AUC of carvedilol with dose increment is mainly a consequence of dose-dependent increase of Vd_{ss} and clearance, respectively (Figures 2 and 3), saturation of carvedilol plasma protein binding could be involved in the non-linear pharmacokinetic pattern. In a previous study, we have found that the unbound fraction of carvedilol increases at higher plasma carvedilol concentrations explaining enhanced tissue distribution of the drug and its non-linear pharmacokinetic behaviour.

In addition, our results suggest that carvedilol pharmacokinetics seems to be affected by the hypertensive stage induced by fructose feeding. Whilst clearance of both enantiomers of carvedilol was not affected by fructose feeding, volume of distribution of R- and S-carvedilol was significantly reduced in fructose hypertensive rats with regards to control normotensive animals after iv application of carvedilol 3 mg/kg. Nevertheless, the mechanisms involved in this finding are unclear.

The main objective of our work was to study enantioselective PK–PD modeling of the carvedilol cardiovascular response in control normotensive and fructose hypertensive rats. Pharmacokinetic–pharmacodynamic (PK–PD) modeling of antihypertensive drugs in animal models of hypertension is a powerful tool to understand underlying pathological mechanisms of different types of hypertension and to refine knowledge of pharmacological properties of blood pressure lowering drugs (Höcht et al., 2008; Bertera et al., 2009). In this way, using a PK–PD modeling approach, we have previously shown the compromise of the vascular sympathetic nervous system in the maintenance of the hypertensive stage in L-NAME rats (Di Verniero et al., 2010).

During PK–PD modeling of cardiovascular effects of carvedilol, it is important to take into account that enantiomers of beta blockers differ regarding their affinity to adrenergic receptors. Whilst only S-carvedilol blocks with high affinity both β_1- and β_2-adrenoceptors, both R and S-carvedilol show similar binding

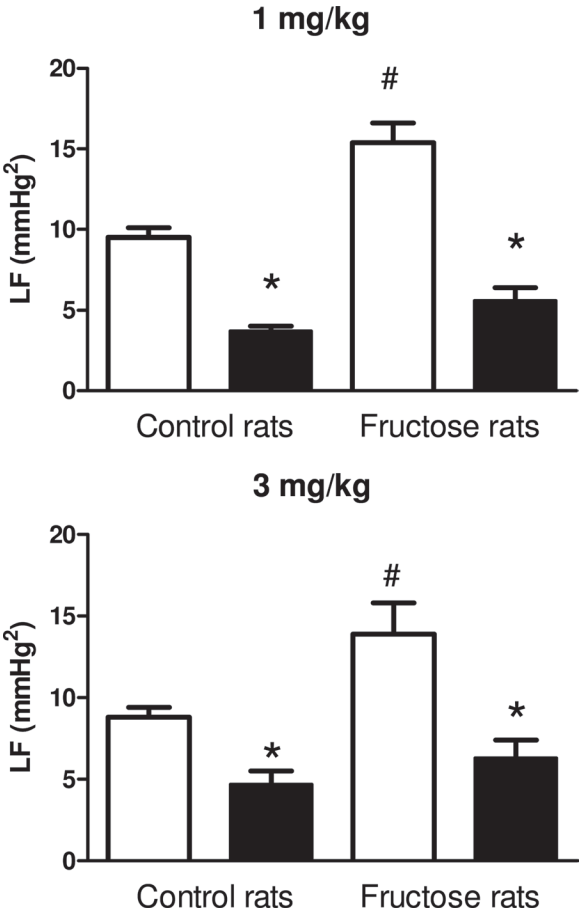

Figure 7. Mean low frequency (LF) variability of blood pressure in control and fructose-fed rats at baseline and after 30 min of carvedilol administration. Each bar shows the mean ± SEM of six rats.

properties to α_1-adrenergic receptors (Bartsch et al., 1990). Therefore, only S-carvedilol plasma concentrations were related to the change in HR in the PK–PD of racemic carvedilol chronotropic effects. Conversely, both enantiomers block α-adrenoceptors with similar affinity, contributing to the hypotensive response to carvedilol. Moreover, the hypotensive activity of the S-enantiomer and the racemate of carvedilol do not differ markedly (Ruffolo et al., 1990), and therefore racemic carvedilol plasma concentrations were used for PK–PD modeling of the drug effects on blood pressure. Finally, as reduction in sympathetic vascular tone is a consequence of α-adrenergic blockade, the sum of S- and R-carvedilol plasma concentrations was used for PK–PD analysis of carvedilol effect on sympathetic vascular tone.

Comparison of PK–PD parameters for the S-carvedilol chronotropic response showed that the hypertensive stage induced by fructose feeding did not change the efficacy and potency of the bradychardic response to carvedilol considering that E_{max} and EC_{50} estimation was similar for control and fructose rats at both dose levels. PK–PD properties of chronotropic response to S-carvedilol were previously studied in L-NAME hypertensive rats (Di Verniero et al., 2010). Compared with findings of the present study, estimated PK–PD parameters (EC_{50}, E_{max} and γ) were in the similar range comparing fructose-fed and L-NAME hypertensive rats (Di Verniero et al., 2010). Moreover, in the previously report PK–PD analysis demonstrated that efficacy and potency of carvedilol effect on the heart rate was not affected by the hypertensive stage induced by L-NAME administration (Di Verniero et al., 2010).

Our findings suggest that this model of metabolic syndrome did not induce overactivity of cardiac sympathetic nervous system or alter activity of cardiac β-adrenoceptor. The results of the present work are in agreement with the fact that the *in vitro* responsiveness to agonist stimulation with noradrenaline or to the inhibition with the inverse agonist metoprolol is not affected in isolated atria from fructose-fed rats (Di Verniero et al., 2008). In addition, although baseline heart rate has several limitations as a marker of cardiac sympathetic activity (Grassi, 1998), the fact that baseline heart rate in fructose rats was not different from control normotensive rats supports the lack of changes in cardiac sympathetic tone and PK–PD properties of the chronotropic response to S-carvedilol.

Regarding assessment of the hypotensive response to carvedilol, time profile of hypotensive response to carvedilol showed significant greater effect of the beta blocker in hypertensive fructose rats compared with normotensive animals. Although the enhanced pharmacodynamic response to carvedilol in fructose rats could be related to greater carvedilol levels, PK–PD analysis have demonstrated a significant greater hypotensive efficacy (E_{max}) of carvedilol in hypertensive rats with regards to control normotensive animals. Conversely, no significant changes were found in EC_{50} and γ for hypotensive response to carvedilol comparing control and fructose-fed animals. Potency and Hill coefficient of carvedilol in fructose rats were similar to those previously found in L-NAME hypertensive animal (Di Verniero et al., 2010). Moreover, an increased hypontensive efficacy of carvedilol was also documented in this experimental model of hypertension (Di Verniero et al., 2010). Therefore, our results suggest that the mechanisms involved in the antihypertensive response to carvedilol are increased in fructose-fed rats.

PK–PD analysis showed an enhancement of the hypotensive response to carvedilol in fructose-fed rats compared with control rats. Efficacy of the blood pressure lowering effect of racemic carvedilol was greater in the hypertensive group only after administration of the higher dose, suggesting that the mechanism of the hypotensive action of carvedilol is enhanced in this model of metabolic syndrome.

Identification of the frequency components of blood pressure variability by power spectral analysis can potentially provide information on mechanisms involved in blood pressure regulation (Stauss, 2007). In this context, renin–angiotensin system peptides,

catecholamines, endothelial-derived NO and myogenic vascular function affect blood pressure variability at VLF (Stauss, 2007). Conversely, LF variability is affected by sympathetic modulation of vascular tone and endothelial-derived NO in rats (Stauss, 2007). Moreover, normalized LF (LF/HF ratio) has been validated as a marker of sympathetic vascular activity in preclinical and clinical studies (Fazan et al., 2008; Souza et al., 2008). Our results showed greater blood pressure variability in the VLF and LF range in fructose-fed rats when compared with control normotensive rats, suggesting a compromise of different endogenous systems, including the renin–angiotensin system, NO and myogenic vascular function, in the regulation of blood pressure. Conversely, LF/HF ratio was not increased in fructose-fed rats compared to normotensive animals indicating the absence of vascular sympathetic overactivity in this experimental model of metabolic syndrome.

Tran et al. (2009) recently reviewed the pathophysiological mechanism involved in the rise of blood pressure in fructose-fed rats stating out that several causative mediators participates in the pathogenesis of fructose-induced hypertension, including the induction of oxidative stress with reduced NO bioavailability, activation of the renin–angiotensin system and sympathetic outflow and blunted vasodilatation to insulin (Tran et al., 2009). The results of our study, using estimation of blood pressure variability

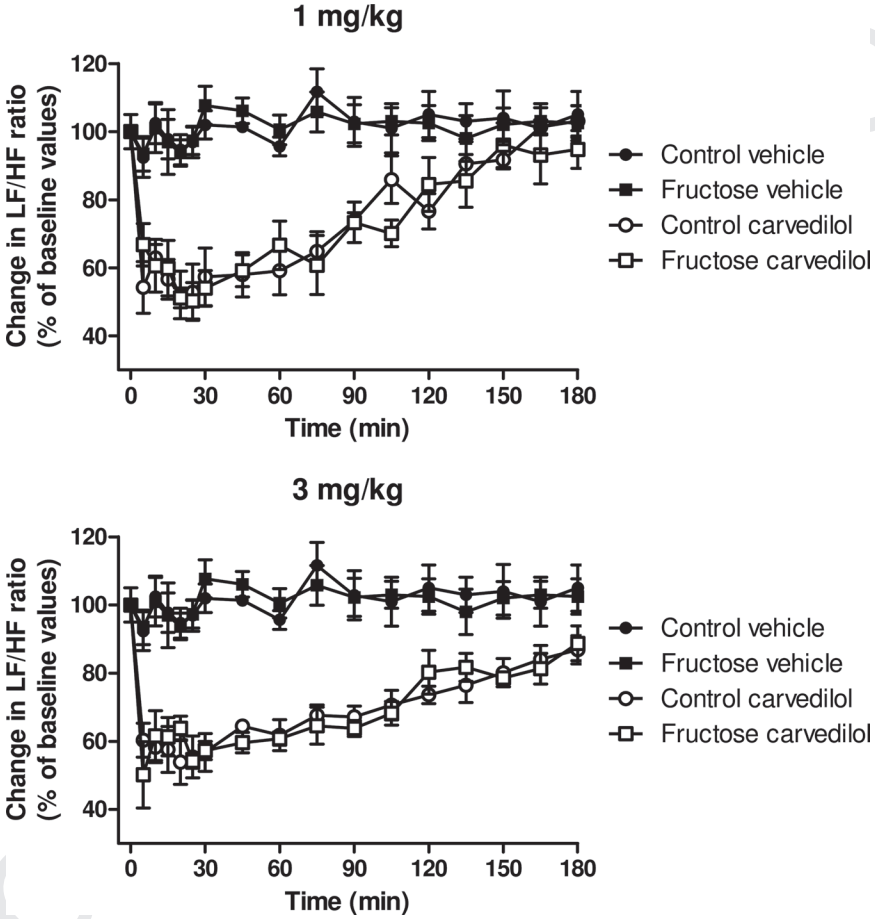

Figure 8. Time course of changes in normalized low frequency (LF) variability (LF/HF ratio), expressed as % of baseline values, after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats.

Table 5. Resulting pharmacokinetic–pharmacodynamic parameters from carvedilol effect on sympathetic vascular activity in control rats and fructose-fed animals after i.v. administration of drug (1 and 3 mg.kg^{-1}): IC_{50}: concentration yielding half maximal inhibition, K_{in}: production rate of the measured response.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg(n=6)	3 mg/kg(n=6)	1 mg/kg(n=6)	3 mg/kg(n=6)
K_{in} (min^{-1})	32.4 ± 7.8	28.9 ± 5.6	22.5 ± 6.9	22.7 ± 4.2
IC_{50} (ng/ml)	1128 ± 166	1304 ± 109	1295 ± 116	1506 ± 198
r^2	0.912 (0.792–0.971)	0.905 (0.815–0.956)	0.902 (0.810–0.972)	0.907 (0.772–0.943)
AIC	87.4 (77.7–90.4)	67.1 (55.2–80.9)	85.9 (74.2–90.8)	65.7 (25.5–94.9)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).

with power spectral analysis, are mainly in agreement with these previous findings, although we did not find an increase in vascular sympathetic activity. Indirect evidence has found sympathetic overactivity in fructose-fed rats. Specifically, it was found that chemical sympathectomy prevents the development of hyperinsulinemia and hypertension and fructose-fed rats shows an increase in urinary excretion of catecholamines (Verma et al., 1999; Kamide et al., 2002). To the best our knowledge, activity of vascular sympathetic nervous system was not previously evaluated in vivo. Therefore, our results suggest that vascular sympathetic nervous system did not play a key role in the maintenance of the hypertensive stage after fructose feeding during 6 weeks.

As comment previously, carvedilol exhibits different pharmacological properties contributing to its antihypertensive effect, including non-specific blockade of β_1- and β_2-adrenoceptors, antagonism of vascular α_1-adrenergic receptors and antioxidant activity. In this context, carvedilol has been shown to possess both reactive oxygen species scavenging and suppressive effect reducing thereby oxidative stress and improving endothelial function. Therefore, it will be interesting to elucidate which mechanism contributes to the increased hypotensive efficacy of carvedilol in fructose-fed rats.

A significant reduction in blood pressure variability in the VLF and LF range was found after carvedilol application in both experimental groups. Moreover, although VLF and LF BPV was significantly increased in fructose rats compared to control animals at baseline, after carvedilol administration no difference were found in BPV in these frequency domains comparing both experimental groups. It is important to mention that carvedilol effect on VLF and LF variability is independent of its hypotensive response, considering that the reduction in blood pressure did not modify its variability in the LF domain (Ponchon et al., 1997). Considering the mechanism of action of carvedilol, our findings using power spectral analysis of arterial pressure recording suggest that carvedilol exhibit increased hypotensive response in fructose-fed rats as a consequence of a greater inhibition of vascular sympathetic activity or reduction of oxidative stress due to its antioxidant properties.

Considering the acceptance of the LF/HF ratio as a marker of sympathetic vascular activity (Fazan et al., 2008; Souza et al., 2008), we evaluated the effect of carvedilol administration on the LF/HF ratio by means of PK–PD modeling in control and fructose treated rats. For the PK–PD analysis of the effects of carvedilol on the LF/HF ratio, an inhibitor indirect physiological PK–PD model with maximal inhibition was used. We assumed that carvedilol can fully inhibit K_{in} in terms of vascular tone considering that, in this experimental work, carvedilol achieves nearly complete suppression of LF variability after administration of the higher dose. These findings are similar to those reported by Ponchon & Elghozy (1997), who found that a subpressor dose of prazosin (α-blocker) reduced LF variability by 72–78%. From a physiological point of view, as LF variability depends on sympathetic tone, it is expected that complete blockade of vascular α-receptors suppressed blood pressure variability in the LF domain (Ponchon et al., 1997; Stauss, 2007).

Comparison of PK–PD parameters obtained from both experimental groups showed that the IC_{50} of carvedilol in fructose rats was not different from control normotensive animals suggesting a similar sympatholytic activity of carvedilol in fructose and control animals. Therefore, considering the absence of an increased *in vivo* blocking activity of carvedilol on β- and α-adrenoceptors in fructose rats, it could be speculated that the increased hypotensive response to carvedilol observed in fructose hypertensive rats results from a greater enhancement of endothelial function due to its antioxidant activity.

In conclusion, carvedilol shows enantioselective pharmacokinetic properties after intravenous administration in control and fructose hypertensive rats. Over a dose range of 1–3 mg/kg, a non-linear pharmacokinetic pattern was described in both experimental groups mainly due to an increase in volume of distribution. In addition, fructose feeding alters pharmacokinetic properties of carvedilol mainly due to an increase in volume of distribution. Enantioselective PK–PD analysis of S-carvedilol effects on HR demonstrated that the beta blocker activity of carvedilol is not affected in fructose hypertensive rats. The hypotensive response to carvedilol is enhanced in fructose-fed animals with regard to control normotensive rats, although reduction of heart rate and vascular sympathetic activity was similar comparing both experimental groups. Although further studies are needed, these results suggest that other mechanisms involved in the antihypertensive response of carvedilol (e.g. antioxidant activity) are enhanced in fructose rats with regards to control animals.

***Declaration of interest*:.**

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Research Article

Recto: Pharmacokinetic–pharmacodynamic modeling of carvedilol

Verso: F. Bertera et al.

Pharmacokinetic and pharmacodynamic properties of carvedilol in fructose hypertensive rats

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Abstract

1. Cardiovascular effects and pharmacokinetics of carvedilol were assessed in fructose-fed rats using pharmacokinetic–pharmacodynamic (PK–PD) modeling.

2. Male Sprague–Dowley rats were randomly assigned to receive tap water (C rats) or fructose solution (10% w/v) (F rats) during 6 weeks. Effects of carvedilol (1–3 mg/kg i.v.) on blood pressure, heart rate and blood pressure variability were recorded. Carvedilol plasma pharmacokinetics was studied by traditional blood sampling. Relationship between carvedilol concentrations and their hypotensive and bradycardic effects was established by PK–PD modeling. Vascular sympatholytic activity of carvedilol was assessed by estimation of drug effects on low frequency blood pressure variability using spectral analysis.

3. A greater volume of distribution and clearance of S-carvedilol compared to R-enantiomer was found in both experimental groups. Although PK–PD properties of S-carvedilol chronotropic effect were not altered in F rats, hypertensive rats showed greater efficacy to the carvedilol hypotensive response after administration of the higher dose. A similar potency of carvedilol to inhibit sympathetic vascular activity was found in F rats.

4. Carvedilol showed enantioselective pharmacokinetic properties with increased distribution in F rats compared with normotensive animals. An enhanced hypotensive activity of carvedilol was found in F rats compared with C rats, which is not related to enhance sympatholytic activity.

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Keywords: Carvedilol, enantioselective pharmacokinetics, hypertension, PK–PD modeling, sympathetic vascular activity, metabolic syndrome, fructose

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Introduction

The metabolic syndrome is a clustering of metabolically related cardiovascular risk factors, including insulin resistance, abdominal obesity, elevated blood pressure, and lipid abnormalities (Alberti et al., 2006). Although the exact mechanism involved in the physiopathology of the metabolic syndrome is actually unknown, increased sympathetic drive seems to play a role in the development of several components of this pathology, such as visceral obesity, high blood pressure, and insulin resistance (Grassi et al., 2004; Grassi et al., 2006). In addition, several preclinical and clinical evidences have demonstrated that blood pressure variability (BPV) is an independent risk factor for the incidence of cardiovascular events associated to hypertension (Su et al., 2005; Höcht et al., 2010). Moreover, reduced heart rate variability and changes in blood pressure variation are nowadays accepted as contributors to cardiovascular disease in patients with metabolic syndrome (Pikkujämsä et al., 1998; Tentolouris et al., 2008). Therefore, treatment of hypertension associated to metabolic syndrome must not only reduce blood pressure levels but also their variability.

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Beta blockers are an attractive therapeutic class for the antihypertensive therapy, considering their cardioprotective effect. Nevertheless, most of beta blockers (e.g. atenolol) negatively affect insulin sensitivity, carbohydrate and lipid metabolism, and are therefore not recommended in metabolic syndrome (Carella et al., 2010). However, recent large studies have shown a better metabolic profile with newer third generation vasodilating beta blockers, including carvedilol and nebivolol, suggesting a possible therapeutic role of these beta blockers in hypertensive patients with metabolic syndrome (Carella et al., 2010).

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Carvedilol is a racemic third generation beta blocker with both enantioselective pharmacokinetic and pharmacodynamic properties (Bartsch et al., 1990; Keating et al., 2006; Prakash et al., 2009). It also shows pleiotropic effects, including antioxidant activity, inhibition of apoptosis, anti-inflammatory action and mitochondrial protection (Ruffolo et al., 1990). Carvedilol enantiomers show different pharmacokinetic behaviour in normotensive animals, considering that the volume of distribution and clearance of S-carvedilol are greater with regard to the R-enantiomer (Fujimaki, 1992; Stahl et al., 1993). Carvedilol enantiomers also differ with respect to their affinity to β-adrenergic receptors. Only S-carvedilol blocks with high affinity both β_1- and β_2-adrenoceptors (Keating et al., 2006). Conversely, both R- and S-carvedilol show similar antagonistic properties on α_1-adrenergic receptors (Bartsch et al., 1990). Therefore, it is expected that carvedilol enantiomers contribute in a different manner to the chronotropic and the hypotensive response.

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Although the pharmacokinetic and pharmacodynamic properties of carvedilol have been investigated in normotensive animals (Bartsch et al., 1990; Ruffolo et al., 1990; Fujimaki, 1992; Stahl et al., 1993), to the best our knowledge, studies regarding the impact of the hypertensive state in experimental models of metabolic syndrome on enantioselective pharmacological behaviour of carvedilol are lacking. The fructose-fed rat is an animal model commonly used to study the association between hypertension and metabolic disorders (Hwang et al., 1987; Catena et al., 2003; Hsieh, 2005). Fructose-fed rat mimics the hypertensive stage associated to the metabolic syndrome and develops an insulin resistance syndrome with a very similar metabolic profile to the human condition, including hyperinsulinemia, insulin resistance, hypertriglyceridemia, and decreased HDL cholesterol (Hwang et al., 1989; Tran et al., 2009).

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Therefore, by using of enantioselective pharmacokinetic–pharmacodynamic (PK/PD) modeling, the aim of the present work was the extensive assessment of the in vivo cardiovascular properties of carvedilol racemics, including the effects on heart rate, blood pressure regulation and its action on short-term blood pressure variability.

Materials and methods

Animals and induction of hypertension

Male Sprague–Dawley rats were used (220–250 g). Animal experiments were performed in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-3, revised 1985). Animals were maintained on a 12-h light/dark cycle. Rats were kept in a room at 22 ± 2ºC and the air was adequately recycled. All animals were fed standard rodent diet (Asociación Cooperativas Argentinas, Buenos Aires, Argentina) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals, and 69% starch and vitamin supplements, containing the same amount of calories.

Rats were randomly divided into two groups: control (n = 18) with tap water to drink for 6 weeks and fructose treated (n = 18) with fructose solution (10% w/v) to drink for 6 weeks. Rats were weighed previously to dietary manipulation and at the end of study. At week 5, blood samples were collected from the retroocular plexus in fasting conditions (5 h), and centrifuged at 4°C. Plasma glucose and triglyceride levels were measured by means of spectrophotometry (Automatic Analyzer Abbott Spectrum CCX, Abbott diagnostics, Abbott Park, IL, USA) and commercial kits (Wiener Glycemia and TG Color GPO/PAP AA, enzymatic methods, Wiener Labs S.A.I.C, Rosario, Argentina).

Preparation of carvedilol formulation

Carvedilol (Droguerías Saporiti, Buenos Aires, Argentina; purity: 100.1%) is practically insoluble in water and therefore a special formula was prepared to allow intravenous administration of the drug at a dose of 1 and 3 mg/kg. The formula of carvedilol solution consisted of 0.1% or 0.3% (w/v) carvedilol, 0.5% (w/v) polyvinylpyrrolidone, 40% (v/v) propylene glycol, 10% (v/v) glycerine and purified water.

Experimental design

Rats were anaesthetized with ether and the left carotid artery and left femoral vein were cannulated with polyethylene cannulae containing heparinized saline solution (25 U/ml). Cannulae were tunneled under the skin and externalized at the back of the neck. Experiments were performed in freely moving animals 24 h after cannulae placement.

The day of the experiment, arterial cannulae was connected to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA) coupled to a Grass 79D polygraph (Grass Instrument, Quincy, MA). The polygraph was connected to a digital converter adaptor unit (Polyview, PVA 1, Grass-Astro Med, West Warwick, RI), and recordings were stored and analyzed with a software program (Polyview 2.3 Astro-Med). Baseline mean arterial pressure (MAP) and heart rate (HR) were estimated during an interval of 60 min. MAP was calculated as the

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sum of the diastolic pressure and one-third of the pulse pressure. HR was estimated tachographically by counting the pulsatile waves of arterial pressure recording.

Carvedilol, at a dose of 1 (n = 6 per group) and 3 mg/kg (n = 6 per group), or vehicle (n = 6 per group) were injected intravenously during 30 s in fructose and control rats. After carvedilol administration, MAP and HR were continuously recorded and blood samples (100 µl) were collected from the arterial cannulae at the following time points: 5, 10, 15, 30, 60, 90, 120 and 180 min.

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Analytical determination of carvedilol

Arterial blood samples (100 µl), collected in polypropylene microcentrifuge tubes containing 5 µl of heparinized solution, were centrifuged at 10,000 rpm for 10 min under controlled temperature (4°C). It is important to mention that blood sampling could alter pharmacokinetic and pharmacodynamic behaviour of antihypertensive drugs due to fluid loss. Nevertheless, in our experimental protocol we only extracted ~800 µl of blood during 3 h period for estimation of plasma concentration of carvedilol. This volume is significantly lower than the recommended maximal volume of blood to be removed (3.5 ml) in a rat weighing 250 g (Aimone, 2005), and therefore it could be suggested that blood loss during our experimental protocol did not affect PK–PD properties of carvedilol.

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Plasma supernatant (30 µl) was carefully separated and carvedilol was extracted by liquid procedure. Briefly, an aliquot of internal standard (2 µg/ml propranolol in methanol), 0.50 M sodium bicarbonate (50 µl) and dichloromethane (1 ml) were added to 30 µl of plasma sample. The mixture was vortexed for 2 min and centrifuged at 2000 rpm for 10 min. The organic layer was transferred into a conical tube and evaporated under nitrogen gas. The dry extract was reconstituted with 100 µl of mobile phase and injected into the chromatographic system.

Levels of R- and S-carvedilol in plasma samples were measured by normal phase liquid chromatography with fluorescence detection using a chiral column (Chirex (S)-ICA and (R)-NEA, Phenomenex) and a fluorescence detector (FL-3000, Thermo Finnigan, France) as described previously (Di Verniero et al., 2010). Briefly, the excitation and emission wavelengths used were 238 and 350 nm, respectively. Optimal composition of the mobile phase was achieved by a mixture of hexane: dichloromethane: ethanol: trifluoroacetic acid (65: 30: 5: 0.2). Retention time of R-carvedilol and S-carvedilol in our chromatographic conditions was 12.8 ± 0.3 min and 14.6 ± 0.4 min, respectively. Coefficient of variation of the chromatographic method was less than 5% and limit of quantification of R- and S-carvedilol was 20 ng.ml^{-1}. The intraday and interday coefficients of variation were 2.8% and 4.5%, respectively. The method was linear in the range of 20–1000 ng.ml^{-1} and samples with higher concentration of carvedilol were diluted with blank plasma in order to achieve concentrations within the validation range.

Estimation of blood pressure variability

Blood pressure variability was continuously estimated by determination of standart deviation and spectral analysis of 3 min periods of blood pressure recordings obtained from baseline and during regular times after carvedilol administration when the quality of the arterial blood pressure signal was visually considered to be satisfactory. According to previous work by other authors (Pladys et al., 2004), spectral analysis of the data was

performed using the Fast Fourier Transform algorithm with a Hamming window (Polyview 2.3 Astro-Med). Spectral densities in the very low frequency range (VLF) (0.1–0.2 Hz), in the low frequency (LF) range (0.2–0.7 Hz), and in the high frequency range (HF) (0.7–2.5 Hz) were calculated (Pladys et al., 2004). Although LF variability is affected by sympathetic modulation of vascular tone, we used LF/HF ratio as an index of vascular sympathetic activity. The normalization procedure tends to minimize the effect of the changes in total power on the absolute values of LF variability (Pladys et al., 2004; Souza et al., 2008).

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Pharmacokinetic–pharmacodynamic analysis

Pharmacokinetics of total R- and S-carvedilol concentrations was estimated by applying a two-compartment, first-order elimination model. Non-linear least squares regression analysis was performed using the TOPFIT program (version 2.0, Dr. Karl Thomae Gmbh, Schering AG, Gödecke AG, Germany) that uses a cyclic three-stage optimization routine (one-dimensional direct search; vectorial direct search/Hooke–Jeeves modified; Gauss–Newton/Marquadt modified). Pharmacokinetic parameters were estimated using both micro and macroconstants. No weighing scheme was used during pharmacokinetic parameter estimation. The area under the curve (AUC) of carvedilol levels vs. time (from 0 to infinity) was calculated using the linear trapezoidal rule. AUC_{0-180} was assessed by subtracting C_{180}/β from$AUC_{0-\infty}$, where C_{180} is the carvedilol concentration at 180 min after drug administration and β the terminal elimination rate constant. Clearance (Cl) and steady state volume of distribution (Vd_{ss}) were calculated by standard methods (Gibaldi et al., 1982).

In the PK–PD relationship study of carvedilol, racemic carvedilol concentrations and S-carvedilol levels were related to blood pressure lowering and chronotropic response to carvedilol, respectively. Relative hypotensive and bradychardic response to carvedilol, expressed as percentage of reduction with regards to baseline values, was estimated at regular times by relating reduction in MAP and HR values to baseline MAP and HR during 30 min before drug administration.

In each experimental subject, pharmacokinetic and pharmacodynamic data were fitted simultaneously for estimation of carvedilol PK–PD parameters. As a time delay between carvedilol plasma concentrations and their cardiovascular effects was observed, a PK–PD model with a separated effect compartment was used for analysis of the data. In previous studies we have found a good correlation between the cardiovascular effects of carvedilol and their plasma levels by the application of PK–PD model with an effect compartment (Bertera et al., 2009; Di Verniero et al., 2010).

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In each experimental subject, a non-linear regression of these data was carried out using the ADAPT II software package (D´Argenio et al., 1997) by means of the sigmoidal Emax equation:

$$Y = \frac{E_{max} * C_e(t)^{\gamma}}{EC_{50} + C_e(t)^{\gamma}}$$

where Y is the change in blood pressure or heart rate expressed as % of basal value, E_{max} is the maximal response, EC_{50} is the carvedilol concentration yielding half maximal response, γ the coefficient of Hill and $C_e(t)$ is the carvedilol concentration (S-carvedilol for the chronotropic response and RS-carvedilol for the hypotensive effect) in the effect compartment at t time. Unweight data were used during PK–PD analysis.

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The following parameters of the PK–PD model were evaluated: EC_{50}, E_{max}, γ and $t_{1/2eq}$. The parameter $t_{1/2eq}$ is the equilibration half time between the plasma and the effect compartment and may be calculated from $\ln 2/k_{e0}$. As reduction of vascular sympathetic activity of carvedilol is related to blockade of α_1-adrenoceptor, RS-carvedilol plasma concentrations were related to LF/HF ratio in order to establish PK–PD properties of the drug on sympathetic activity on the vascular system. In a previous work, we have found a good correlation between carvedilol plasma concentrations and the effect on LF/HF ratio by using a physiological indirect PK–PD model. Birefly, we assumed that the vascular sympathetic activity (LF/HF ratio) is produced constantly through a zero order kinetics (K_{in}) and removed in a first order kinetics with a rate constant K_{out} (Di Verniero et al., 2010). Carvedilol inhibits the production of the sympathetic tone (inhibition of K_{in}) thereby affecting its magnitude. In each experimental subject, effects of carvedilol on vascular sympathetic activity were related to drug levels in the central compartment by means of the following equation:

$$dR/dt = K_{in}\left[1 - \frac{C_c}{C_c + IC_{50}}\right] - K_{out}R$$

where dR/dt is the change in LF/HF ratio, C_c the racemic carvedilol concentration in the central compartment and IC_{50} is the drug concentration that produces 50% of vascular sympathetic tone inhibition. K_{out} was fixed as the function of K_{in} and the baseline response ($K_{out} = K_{in}/R_0$). PK–PD analysis of the data was carried out using the ADAPT II software package (D'Argenio et al., 1997). Unweight data were used during PK–PD analysis.

Statistical analysis

Normal distribution of the data and the variables of the study were verified using the Kolmogorov–Smirnov test. Data were expressed as means ± SEM. Basal values of MAP, HR and LF/HF ratio were compared by means of Student's *t* test. Statistical analysis of carvedilol effects on MAP, HR and LF/HF ratio was performed by two-way analysis of variance (ANOVA) and the test of Bonferroni as *post hoc* test. Pharmacokinetic and PK–PD parameters were log transformed for statistical analysis in order to reduce heterogeneity of the variance and further compared by two-way ANOVA and the test of Bonferroni as *post hoc* test. Correlation between maximal plasma concentration (C_{max}):Dose ratio or AUC:Dose ratio and other pharmacokinetics parameters (Vd_{ss} and Cl) was studied by means of Pearson's test. Statistical tests were performed using GraphPad Prism version 5.02 for Windows (GraphPad Software, San Diego, California, CA). Statistical significance was defined as $p < 0.05$.

Results

Basaline values of glycemia, triglyceridemia, MAP and HR in control and fructose rats are shown in Table 1. Compared with normotensive animals, fructose feeding increased glycemia, triglyceridemia and MAP without changing HR (Table 1). These results are in agreement with metabolic and hemodynamic profiles previously reported in this experimental model of metabolic syndrome (Hsieh, 2005; Mayer et al., 2007; Mayer et al.; 2008).

Carvedilol pharmacokinetics

Figure 1 shows the concentration-time profile of S-carvedilol and R-carvedilol plasma concentrations in control rats and fructose hypertensive rats after intravenous administration of 1 (n = 6 for each group) and 3 mg/kg (n = 6 for each group) of the drug. A biexponential decay of plasma carvedilol levels was found in all experimental groups compatible with a pharmacokinetic two-compartment model (Figure 1). The resulting pharmacokinetic parameters are shown in Table 2. No differences were found in constant of distribution and constant of elimination comparing all experimental groups.

A dose-dependent increase in the volume of distribution of both carvedilol enantiomers was found in normotensive control rats. Conversely, only volume of distribution of R-carvedilol showed dose dependency in fructose-fed rats. After administration of racemic carvedilol 3 mg/kg, the volume of distribution of S- and R-carvedilol was significantly reduced in fructose hypertensive animals compared to normotensive group. In addition, although clearance of both enantiomers was not affected by the hypertensive stage induced by fructose feeding, S-carvedilol clearance showed a dose-dependent increase in control rats (Table 2). As a consequence of the dose dependence of the volume of distribution and clearance estimations, both maximal plasma concentration and AUC increased less than proportionally for both R- and S-Carvedilol in control rats and for R-isomer in fructose animals (Table 2). Confirming these results, C_{max} and AUC of both R- and S-carvedilol showed a significant negative correlation with Vd_{ss} and Cl, respectively (Figures 2 and 3).

PK–PD modeling of the carvedilol chronotropic effect

Figure 4 shows HR changes time profile in control and fructose rats after vehicle or carvedilol intravenous administration at a dose of 1 and 3 mg/kg. Vehicle administration (n = 6 for each group) did not modify HR in either experimental group (Figure 4). The chronotropic response to carvedilol was not significantly different comparing Fructose feeding rats (–20.4 ± 3.2% for 1 mg/kg, n = 6; –30.4 ± 3.7% for 3 mg/kg, n = 6) with Control animals (–18.9 ± 2.3% for 1 mg/kg, n = 6; –24.8 ± 1.5% for 3 mg/kg, n = 6) after administration of both doses.

When correlating the chronotropic response to S-carvedilol concentrations, an effect compartment PK–PD model with sigmoidal E_{max} equation fitted well in all experimental groups (Table 3). No differences were found in E_{max} estimation comparing both dose levels in control and fructose animals (Table 3), suggesting that the complete pharmacodynamic range of carvedilol bradycardic effect was attained under our experimental conditions.The rate of carvedilol distribution at the biophase did not differ when comparing all experimental groups (Table 3). In addition, maximal chronotropic response and potency of S-carvedilol were similar comparing fructose-fed rats and normotensive control rats.

PK–PD modeling of the carvedilol hypotensive effect

Figure 5 shows the MAP changes time profile in control and fructose-fed animals after vehicle or carvedilol intravenous administration at a dose of 1 and 3 mg/kg. Vehicle administration (n = 6 for each group) did not modify blood pressure in either experimental group (Figure 5). The hypotensive response to carvedilol was significantly greater in fructose feeded rats (–30.3 ± 2.6%, n = 6, $p < 0.05$) compared with control rats (–19.6 ± 1.7%, n = 6) after intravenous administration of carvedilol 3 mg/kg.

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When correlating the blood pressure lowering response to racemic carvedilol concentrations, the effect compartment PK–PD model with sigmoidal Emax equation fitted well in all experimental groups. No differences were found in E_{max} estimation comparing both dose levels in control and fructose hypertensive rats (Table 4), suggesting that the complete pharmacodynamic range of the carvedilol hypotensive effect was attained under our experimental conditions and the sigmoidal Emax equation is suitable for PK–PD parameter estimation.

Rate transfer of carvedilol from the central to the effect compartment did not differ in all experimental groups (Table 4). The maximal hypotensive response was significantly greater in fructose-fed rats compared with normotensive control rats. Potency of carvedilol hypotensive response was similar comparing both experimental groups with a non-significant increase of EC_{50} with dose increment (Table 4).

Effect of carvedilol on blood pressure variability and vascular adrenergic tone

Fructose hypertensive rats showed increased blood pressure variability compared with control rats. While both VLF and LF variability was greater in Fructose rats (VLF 19.0 ± 1.9 mmHg2; LF 12.8 ± 1.3 mmHg2; $n = 18$, $p < 0.05$) compared with the normotensive group (VLF 12.7 ± 1.0 mmHg2; LF 9.1 ± 0.5 mmHg2; $n = 18$) (Figures 6 and 7), no difference was found in HF variability between experimental groups (Control rats: 3.2 ± 0.3 $mmHg^2$, $n = 18$; Fructose: 4.1 ± 0.5 $mmHg^2$, $n = 18$). Vascular sympathetic activity estimated by baseline LF/HF ratio was not significantly different in fructose hypertensive rats (3.5 ± 0.2, $n = 18$) compared to normotensive control animals (3.2 ± 0.2, $n = 18$).

On the other hand, carvedilol administration significantly reduced BPV in the VLF and LF domain in control and fructose rats (Figures 6 and 7) without affecting HF variability of blood pressure in both experimental groups (data not shown). Vehicle administration ($n = 6$ for each group) did not modify blood pressure variability in fructose and control rats (data not shown).

Figure 8 shows the LF/HF ratio—time profile after vehicle or carvedilol (1 and 3 mg/kg) administration in control ($n = 6$ for each dose level) and fructose-fed rats ($n = 6$ for each dose level). While vehicle administration did not modify the LF/HF ratio in both experimental groups (Figure 8), carvedilol administration induced a similar reduction of this parameter in normotensive control rats and hypertensive frutose animals (Figure 8).

When correlating sympathetic vascular activity expressed as LF/HF ratio to racemic carvedilol plasma concentrations, the inhibitory physiological indirect PK–PD model fitted well in all experimental groups (Table 5). No differences were found in K_{in} and IC_{50} estimation comparing control and fructose rats. Estimation of PK–PD parameters for the carvedilol effect on sympathetic vascular tone did not change with dose increment in either experimental group.

Discussion

This study yielded several findings regarding enantioselective PK–PD properties of carvedilol in fructose hypertensive rats. Carvedilol enantiomers show different pharmacokinetic behaviour, considering that clearance and volume of distribution of S-carvedilol are significantly greater than R-carvedilol. Both enantiomers exhibit

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non-linear pharmacokinetics and the volume of distribution of of S- and R-carvedilol is reduced in hypertensive fructose rats compared with control rats after administration of the higher dose. The hypotensive response to carvedilol is enhanced in fructose-fed animals with regard to control normotensive rats, although reduction of heart rate and vascular sympathetic activity were similar comparing both experimental groups. These results suggest that other mechanisms involved in the antihypertensive response of carvedilol, such as its antioxidant activity, are enhanced in fructose rats with regards to control animals.

Carvedilol pharmacokinetics have been studied previously in both human volunteers (Neugebauer et al., 1990; Zhou et al., 1995; Phuong et al., 2004) and rats (Fujimaki, 1992; Stahl et al., 1993; Di Verniero et al., 2010). Carvedilol enantiomers show high plasma protein binding and metabolize through hepatic cytochrome P450 2D6 and P450 1A2, and intestinal cytochrome P450 3A4 (Ishida et al., 2008). The extraction fraction of carvedilol is high, showing an oral bioavailability of 0.19 and 0.83 in human volunteers and patients with cirrhosis, respectively (Verbeeck, 2008). In addition, several studies have described an enantioselective pharmacokinetic profile of carvedilol enantiomers: S-carvedilol shows a greater volume of distribution, clearance and presystemic elimination with regard to R-carvedilol (Fujimaki, 1992; Stahl et al., 1993; Di Verniero et al., 2010). In agreement with these findings, we found higher values for Vd_{ss} and Cl of S-carvedilol compared with R-carvedilol in both normotensive control rats and hypertensive fructose treated rats.

We studied carvedilol pharmacokinetics 24 h after arterial cannulation in rats. It has been demonstrated that surgical implantation of cannulae 24 h before measurements are taken induced an increment of α_1-glycoprotein (Terao et al., 1983). Although α_1-glycoprotein binds basic drugs, carvedilol binds predominantly to serum albumin (Stahl et al., 1993; Frishman, 1998) and, therefore, it seems unlikely that an increase in α_1-glycoprotein due to cannulae implantation would affect the carvedilol free fraction in our experimental conditions.

The relationship between carvedilol pharmacokinetics and dosing was assessed after administration of 1 and 3 mg/kg of the drug. Linear pharmacokinetics of carvedilol has been described in elderly subjects after oral administration of 25–50 mg of the drug (Louis et al., 1987). Conversely, a saturable first-pass effect for carvedilol was found in rats after high oral racemate dosing (Stahl et al., 1993). Our results suggested that, after application of a single intravenous dose over the range of 1–3 mg/kg, both S- and R-carvedilol showed a non-linear pharmacokinetic pattern in control and, only R-carvedilol, in fructose-fed rats mainly as a consequence of an increased Vd_{ss}. In addition, while both S- and R-carvedilol clearance did not change with dosing in fructose rats, S-carvedilol clearance showed a dose-dependent enhancement in control normotensive rats. A similar non-linear profile of carvedilol pharmacokinetics was previously found in Wistar normotensive rats and N^G-nitro-L-arginine methyl ester (L-NAME) hypertensive animals (Di Verniero et al., 2010). Considering that the lower than expected increase of C_{max} and AUC of carvedilol with dose increment is mainly a consequence of dose-dependent increase of Vd_{ss} and clearance, respectively (Figures 2 and 3), saturation of carvedilol plasma protein binding could be involved in the non-linear pharmacokinetic pattern. In a previous study, we have found that the unbound fraction of carvedilol increases at higher plasma carvedilol concentrations explaining enhanced tissue distribution of the drug and its non-linear pharmacokinetic behaviour. In addition, our results suggest that carvedilol pharmacokinetics seems to be affected by the hypertensive stage induced by fructose feeding. Whilst clearance of both enantiomers of carvedilol was not affected by fructose feeding, volume of distribution of R- and S-carvedilol was significantly reduced in fructose hypertensive rats

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with regards to control normotensive animals after iv application of carvedilol 3 mg/kg. Nevertheless, the mechanisms involved in this finding are unclear.

The main objective of our work was to study enantioselective PK–PD modeling of the carvedilol cardiovascular response in control normotensive and fructose hypertensive rats. Pharmacokinetic–pharmacodynamic (PK–PD) modeling of antihypertensive drugs in animal models of hypertension is a powerful tool to understand underlying pathological mechanisms of different types of hypertension and to refine knowledge of pharmacological properties of blood pressure lowering drugs (Höcht et al., 2008; Bertera et al., 2009). In this way, using a PK–PD modeling approach, we have previously shown the compromise of the vascular sympathetic nervous system in the maintenance of the hypertensive stage in L-NAME rats (Di Verniero et al., 2010).

During PK–PD modeling of cardiovascular effects of carvedilol, it is important to take into account that enantiomers of beta blockers differ regarding their affinity to adrenergic receptors. Whilst only S-carvedilol blocks with high affinity both β_1- and β_2-adrenoceptors, both R and S-carvedilol show similar binding properties to α_1-adrenergic receptors (Bartsch et al., 1990). Therefore, only S-carvedilol plasma concentrations were related to the change in HR in the PK–PD of racemic carvedilol chronotropic effects. Conversely, both enantiomers block α-adrenoceptors with similar affinity, contributing to the hypotensive response to carvedilol. Moreover, the hypotensive activity of the S-enantiomer and the racemate of carvedilol do not differ markedly (Ruffolo et al., 1990), and therefore racemic carvedilol plasma concentrations were used for PK–PD modeling of the drug effects on blood pressure. Finally, as reduction in sympathetic vascular tone is a consequence of α-adrenergic blockade, the sum of S- and R-carvedilol plasma concentrations was used for PK–PD analysis of carvedilol effect on sympathetic vascular tone.

Comparison of PK–PD parameters for the S-carvedilol chronotropic response showed that the hypertensive stage induced by fructose feeding did not change the efficacy and potency of the bradychardic response to carvedilol considering that E_{max} and EC_{50} estimation was similar for control and fructose rats at both dose levels. PK–PD properties of chronotropic response to S-carvedilol were previously studied in L-NAME hypertensive rats (Di Verniero et al., 2010). Compared with findings of the present study, estimated PK–PD parameters (EC_{50}, E_{max} and γ) were in the similar range comparing fructose-fed and L-NAME hypertensive rats (Di Verniero et al., 2010). Moreover, in the previously report PK–PD analysis demonstrated that efficacy and potency of carvedilol effect on the heart rate was not affected by the hypertensive stage induced by L-NAME administration (Di Verniero et al., 2010).

Our findings suggest that this model of metabolic syndrome did not induce overactivity of cardiac sympathetic nervous system or alter activity of cardiac β-adrenoceptor. The results of the present work are in agreement with the fact that the *in vitro* responsiveness to agonist stimulation with noradrenaline or to the inhibition with the inverse agonist metoprolol is not affected in isolated atria from fructose-fed rats (Di Verniero et al., 2008). In addition, although baseline heart rate has several limitations as a marker of cardiac sympathetic activity (Grassi, 1998), the fact that baseline heart rate in fructose rats was not different from control normotensive rats supports the lack of changes in cardiac sympathetic tone and PK–PD properties of the chronotropic response to S-carvedilol.

Regarding assessment of the hypotensive response to carvedilol, time profile of hypotensive response to carvedilol showed significant greater effect of the beta blocker in hypertensive fructose rats compared with

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normotensive animals. Although the enhanced pharmacodynamic response to carvedilol in fructose rats could be related to greater carvedilol levels, PK–PD analysis have demonstrated a significant greater hypotensive efficacy (E_{max}) of carvedilol in hypertensive rats with regards to control normotensive animals. Conversely, no significant changes were found in EC_{50} and γ for hypotensive response to carvedilol comparing control and fructose-fed animals. Potency and Hill coefficient of carvedilol in fructose rats were similar to those previously found in L-NAME hypertensive animal (Di Verniero et al., 2010). Moreover, an increased hypontensive efficacy of carvedilol was also documented in this experimental model of hypertension (Di Verniero et al., 2010). Therefore, our results suggest that the mechanisms involved in the antihypertensive response to carvedilol are increased in fructose-fed rats.

PK–PD analysis showed an enhancement of the hypotensive response to carvedilol in fructose-fed rats compared with control rats. Efficacy of the blood pressure lowering effect of racemic carvedilol was greater in the hypertensive group only after administration of the higher dose, suggesting that the mechanism of the hypotensive action of carvedilol is enhanced in this model of metabolic syndrome.

Identification of the frequency components of blood pressure variability by power spectral analysis can potentially provide information on mechanisms involved in blood pressure regulation (Stauss, 2007). In this context, renin–angiotensin system peptides, catecholamines, endothelial-derived NO and myogenic vascular function affect blood pressure variability at VLF (Stauss, 2007). Conversely, LF variability is affected by sympathetic modulation of vascular tone and endothelial-derived NO in rats (Stauss, 2007). Moreover, normalized LF (LF/HF ratio) has been validated as a marker of sympathetic vascular activity in preclinical and clinical studies (Fazan et al., 2008; Souza et al., 2008). Our results showed greater blood pressure variability in the VLF and LF range in fructose-fed rats when compared with control normotensive rats, suggesting a compromise of different endogenous systems, including the renin–angiotensin system, NO and myogenic vascular function, in the regulation of blood pressure. Conversely, LF/HF ratio was not increased in fructose-fed rats compared to normotensive animals indicating the absence of vascular sympathetic overactivity in this experimental model of metabolic syndrome.

Tran et al. (2009) recently reviewed the pathophysiological mechanism involved in the rise of blood pressure in fructose-fed rats stating out that several causative mediators participates in the pathogenesis of fructose-induced hypertension, including the induction of oxidative stress with reduced NO bioavailability, activation of the renin–angiotensin system and sympathetic outflow and blunted vasodilatation to insulin (Tran et al., 2009). The results of our study, using estimation of blood pressure variability with power spectral analysis, are mainly in agreement with these previous findings, although we did not find an increase in vascular sympathetic activity.

Indirect evidence has found sympathetic overactivity in fructose-fed rats. Specifically, it was found that chemical sympathectomy prevents the development of hyperinsulinemia and hypertension and fructose-fed rats shows an increase in urinary excretion of catecholamines (Verma et al., 1999; Kamide et al., 2002). To the best our knowledge, activity of vascular sympathetic nervous system was not previously evaluated in vivo. Therefore, our results suggest that vascular sympathetic nervous system did not play a key role in the maintenance of the hypertensive stage after fructose feeding during 6 weeks.

As comment previously, carvedilol exhibits different pharmacological properties contributing to its antihypertensive effect, including non-specific blockade of β_1- and β_2-adrenoceptors, antagonism of vascular α_1-adrenergic receptors and antioxidant activity. In this context, carvedilol has been shown to possess both reactive

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oxygen species scavenging and suppressive effect reducing thereby oxidative stress and improving endothelial function. Therefore, it will be interesting to elucidate which mechanism contributes to the increased hypotensive efficacy of carvedilol in fructose-fed rats.

A significant reduction in blood pressure variability in the VLF and LF range was found after carvedilol application in both experimental groups. Moreover, although VLF and LF BPV was significantly increased in fructose rats compared to control animals at baseline, after carvedilol administration no difference were found in BPV in these frequency domains comparing both experimental groups. It is important to mention that carvedilol effect on VLF and LF variability is independent of its hypotensive response, considering that the reduction in blood pressure did not modify its variability in the LF domain (Ponchon et al., 1997). Considering the mechanism of action of carvedilol, our findings using power spectral analysis of arterial pressure recording suggest that carvedilol exhibit increased hypotensive response in fructose-fed rats as a consequence of a greater inhibition of vascular sympathetic activity or reduction of oxidative stress due to its antioxidant properties.

Considering the acceptance of the LF/HF ratio as a marker of sympathetic vascular activity (Fazan et al., 2008; Souza et al., 2008), we evaluated the effect of carvedilol administration on the LF/HF ratio by means of PK–PD modeling in control and fructose treated rats. For the PK–PD analysis of the effects of carvedilol on the LF/HF ratio, an inhibitor indirect physiological PK–PD model with maximal inhibition was used. We assumed that carvedilol can fully inhibit K_{in} in terms of vascular tone considering that, in this experimental work, carvedilol achieves nearly complete suppression of LF variability after administration of the higher dose. These findings are similar to those reported by Ponchon & Elghozy (1997), who found that a subpressor dose of prazosin (α-blocker) reduced LF variability by 72–78%. From a physiological point of view, as LF variability depends on sympathetic tone, it is expected that complete blockade of vascular α-receptors suppressed blood pressure variability in the LF domain (Ponchon et al., 1997; Stauss, 2007).

Comparison of PK–PD parameters obtained from both experimental groups showed that the IC_{50} of carvedilol in fructose rats was not different from control normotensive animals suggesting a similar sympatholytic activity of carvedilol in fructose and control animals. Therefore, considering the absence of an increased *in vivo* blocking activity of carvedilol on β- and α-adrenoceptors in fructose rats, it could be speculated that the increased hypotensive response to carvedilol observed in fructose hypertensive rats results from a greater enhancement of endothelial function due to its antioxidant activity.

In conclusion, carvedilol shows enantioselective pharmacokinetic properties after intravenous administration in control and fructose hypertensive rats. Over a dose range of 1–3 mg/kg, a non-linear pharmacokinetic pattern was described in both experimental groups mainly due to an increase in volume of distribution. In addition, fructose feeding alters pharmacokinetic properties of carvedilol mainly due to an increase in volume of distribution. Enantioselective PK–PD analysis of S-carvedilol effects on HR demonstrated that the beta blocker activity of carvedilol is not affected in fructose hypertensive rats. The hypotensive response to carvedilol is enhanced in fructose-fed animals with regard to control normotensive rats, although reduction of heart rate and vascular sympathetic activity was similar comparing both experimental groups. Although further studies are needed, these results suggest that other mechanisms involved in the antihypertensive response of carvedilol (e.g. antioxidant activity) are enhanced in fructose rats with regards to control animals.

Declaration of interest

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Table 1. Baseline metabolic and hemodynamic parameters in control and fructose rats.

Parameter	Control rats (n = 18)	Fructose rats (n = 18)
Glycemia (mg/ml)	1.36 ± 0.04	1.56 ± 0.03*
Triglyceridemia (mg/ml)	0.51 ± 0.07	1.02 ± 0.09*
MAP (mmHg)	105 ± 2	114 ± 2

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HR (bpm)	382 ± 14	377 ± 12

*$p < 0.05$ vs. control rats.

Table 2. Pharmacokinetic parameters of total S-carvedilol and R-carvedilol plasma levels obtained from arterial blood samples: AUC (area under the curve), α (constant of distribution), β (constant of elimination), Cl (clearance) and Vdss (steady state volume of distribution), Cmax (extrapolated maximal concentration) in control rats and fructose treated animals after i.v. administration of drug (1 mg/kg and 3 mg/kg).

Enantiomer	S-carvedilol				R-carvedilol			
Experimental group	Control rats		Fructose rats		Control rats		Fructose rats	
Dose	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)
α (h^{-1})	12.3 ± 1.2	8.0 ± 1.3	14.3 ± 1.4	9.3 ± 1.9	7.8 ± 1.8	6.3 ± 1.3	8.3 ± 1.2	8.0 ± 1.5
β (h^{-1})	0.43 ± 0.15	0.57 ± 0.07§	0.44 ± 0.09	0.38 ± 0.09	0.38 ± 0.19	0.25 ± 0.04	0.47 ± 0.07	0.31 ± 0.08
Vd_{ss} (l)	1.13 ± 0.26	2.24 ± 0.29$^{\#\S}$	1.14 ± 0.11§	1.21 ± 0.07§*	0.77 ± 0.11	1.51 ± 0.09$^{\#}$	0.53 ± 0.03	0.89 ± 0.07*$^{\#}$
Cl ($ml.min^{-1}$)	6.5 ± 1.4	14.2 ± 3.4$^{\#\S}$	9.1 ± 1.0§	8.9 ± 1.6§	4.5 ± 1.2	6.7 ± 1.1	4.4 ± 0.5	4.7 ± 1.3
C_{max} ($\mu g.ml^{-1}$)	1.61 ± 0.09	2.49 ± 0.34	1.64 ± 0.15	3.30 ± 0.26	1.60 ± 0.18	2.77 ± 0.32	2.02 ± 0.32	3.50 ± 0.22
$AUC_{0-\infty}$ ($ng.ml.h^{-1}$)	1675 ± 372	2487 ± 665	1000 ± 141	3802 ± 766*	2447 ± 487	4300 ± 803	2064 ± 296	4273 ± 609*

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).
$^{\#}p < 0.05$ vs. 1 $mg.kg^{-1}$
*$p < 0.05$ vs. Control rats.
$^{\S}p < 0.05$ vs. R-carvedilol.

Table 3. Resulting pharmacokinetic–pharmacodynamic parameters from the chronotropic effect of carvedilol in control rats and fructose-fed treated animals after i.v. administration of drug (1 and 3 mg/kg): EC_{50}: concentration yielding half maximal response, E_{max}: maximal response, γ: coefficient of Hill, $t_{1/2eq}$: equilibration half-life between the plasma and the effect compartment.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)
E_{max} (%)	20.5 ± 2.5	26.5 ± 2.5	22.4 ± 3.4	31.1 ± 3.6
EC_{50}(μg/ml)	0.63 ± 0.11	0.67 ± 0.15	0.72 ± 0.09	0.84 ± 0.15
□	2.2 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.4 ± 0.2
$t_{1/2eq}$ (min)	5.2 ± 1.5	3.2 ± 1.0	4.5 ± 1.2	2.1 ± 0.8
r^2	0.957 (0.922–0.982)	0.938 (0.881–	0.905 (0.839–0.987)	0.945 (0.875–

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		0.985)		0.978)
AIC	62.9 (52.8–76.7)	74.9 (62.5–89.7)	76.6 (55.8-125.8)	68.5 (47.1–93.4)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).

Table 4. Resulting pharmacokinetic–pharmacodynamic parameters from the hypotensive effect of carvedilol in control rats and fructose-fed animals after i.v. administration of drug (1 and 3 mg.kg^{-1}): EC_{50}: concentration yielding half maximal response, E_{max}: maximal response, γ: coefficient of Hill, $t_{1/2eq}$: equilibration half-life between the plasma and the effect compartment.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)
E_{max} (%)	30.6 ± 1.5	24.5 ± 2.1	34.7 ± 3.9	35.0 ± 2.8*
EC_{50}(µg/ml)	0.63 ± 0.11	0.68 ± 0.52	0.72 ± 0.09	0.83 ± 0.14
□	2.2 ± 0.2	2.0 ± 0.1	2.2 ± 0.2	2.4 ± 0.2
$t_{1/2eq}$ (min)	5.5 ± 1.2	4.2 ± 1.4	5.7 ± 1.8	7.6 ± 1.4
r^2	0.955 (0.922–0.982)	0.956 (0.881–0.985)	0.949 (0.839–0.987)	0.985 (0.875–0.978)
AIC	61.5 (51.5–73.2)	64.2 (60.5–85.7)	66.6 (58.8–105.6)	62.5 (47.1–92.6)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).
*p < 0.05 vs Control rats.

Table 5. Resulting pharmacokinetic–pharmacodynamic parameters from carvedilol effect on sympathetic vascular activity in control rats and fructose-fed animals after i.v. administration of drug (1 and 3 mg.kg^{-1}): IC_{50}: concentration yielding half maximal inhibition, K_{in}: production rate of the measured response.

Experimental group	Control rats		Fructose rats	
Dose	1 mg/kg (n = 6)	3 mg/kg (n = 6)	1 mg/kg (n = 6)	3 mg/kg (n = 6)
K_{in} (min^{-1})	32.4 ± 7.8	28.9 ± 5.6	22.5 ± 6.9	22.7 ± 4.2
IC_{50}(ng/ml)	1128 ± 166	1304 ± 109	1295 ± 116	1506 ± 198
r^2	0.912 (0.792–0.971)	0.905 (0.815–0.956)	0.902 (0.810–0.972)	0.907 (0.772–0.943)
AIC	87.4 (77.7–90.4)	67.1 (55.2–80.9)	85.9 (74.2–90.8)	65.7 (25.5–94.9)

Data are expressed as mean ± SEM. Goodness of fit indicators are expressed as mean (range).

Figure 1. Mean plasma concentration values of S-carvedilol and R-carvedilol vs. time in control normotensive rats (circles) and fructose-fed animals (squares) after administration of 1 mg/kg (black symbols) and 3 mg/kg (open symbols) of the drug. Each point shows the mean ± SEM of six rats.

Figure 2. Correlation between maximal plasma concentrarion (C_{max}):Dose ratio of R-carvedilol and S-carvedilol and steady state volume of distribution (Vdss) in control and fructose rats. A significant negative correlation was found between Cmax:Dose ratio and Vd_{ss} for both R-carvedilol (r = -0.5943) and S-carvedilol (r = -0.6280).

Figure 3. Correlation between area under the curve (AUC):Dose ratio of R-carvedilol and S-carvedilol and clearance (Cl) in control and fructose rats. A significant negative correlation was found between AUC:Dose ratio and Cl for both R-carvedilol (r = -0.8708) and S-carvedilol (r = -0.8520).

Figure 4. Time course of changes in heart rate (ΔHR, % of baseline values), after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats. *p < 0.05 vs. control rats.

Figure 5. Time course of changes in mean arterial pressure (ΔMAP, % of baseline values), after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats.

Figure 6. Mean very low frequency (VLF) variability of blood pressure in control and fructose-fed rats at baseline and after 30 min of carvedilol administration. Each bar shows the mean ± SEM of six rats.

Figure 7. Mean low frequency (LF) variability of blood pressure in control and fructose-fed rats at baseline and after 30 min of carvedilol administration. Each bar shows the mean ± SEM of six rats.

Figure 8. Time course of changes in normalized low frequency (LF) variability (LF/HF ratio), expressed as % of baseline values, after i.v. administration of carvedilol 1 mg/kg and 3 mg/kg (open symbols) or vehicle (black symbols) in control normotensive rats (circles) and fructose-fed treated animals (squares). Each point shows the mean ± SEM of six rats.

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