ORIGINAL ARTICLE

THE INFLUENCE OF SERTRALINE ON THE PHARMACOKINETICS OF CARVEDILOL. A PRECLINICAL STUDY ON RATS

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Abstract

The objective of this study was to investigate the effect of multiple-dose sertraline on the pharmacokinetic profile of carvedilol in rats. Carvedilol was orally administrated in rats (3.57 mg/kg body mass (b.m.)) on the absence of sertraline or after a pre-treatment with multiple oral doses of sertraline (7.14 mg/kg b.m.). The plasma concentrations of carvedilol were estimated by HPLC-MS. After carvedilol co-administration with sertraline, an approximately 7.7-fold increase in the exposure of carvedilol was observed, considering the significantly elevated value of $AUC_{0-\infty}$. Moreover, an increase by 250% of peak plasma concentration was found, as well as an augmentation by 450% of the half life time of carvedilol was observed. Sertraline co-administration led to a significant alteration of carvedilol's pharmacokinetic profile in rats, these effects could be explained by the existence of a drug-drug interaction mediated by CYP2D6 inhibition.

Rezumat

Obiectivul studiului a fost acela de a investiga efectul administrării unor doze multiple de sertralină asupra profilului farmacocinetic al carvedilolului la şobolani. Carvedilolul s-a administrat oral (3,57 mg/kg corp) în absenţa sertralinei, precum şi după un tratament prealabil cu doze multiple de sertralină administrate oral (7,14 mg/kg corp). Concentraţiile plasmatice ale carvedilolului s-au determinat prin HPLC-MS. După coadministrarea celor două substanţe medicamentoase, s-a observat o expunere de 7,7 ori mai mare pentru carvedilol. Mai mult, s-a înregistrat o creştere cu 250% a concentraţiei plasmatice maxime şi cu 450% a timpului de înjumătăţire al carvedilolului. Co-administrarea sertralinei a dus la o modificare semnificativă a profilului farmacocinetic al carvedilolului la şobolani, acest efect putând fi explicat de existenţa unei interacţiuni medicamentoase mediate de inhibarea izoenzimei CYP2D6.

Keywords: carvedilol, cytochrome P450s, pharmacokinetics

Introduction

Carvedilol is a nonselective β-blocking agent, which is commonly used for the treatment of different cardiovascular diseases, including angina, heart failure or hypertension [11]. Carvedilol is a third generation β-blocker and possesses some advantages due to its nitric oxide - dependent effects (vaso-dilatation, anti-proliferation and cardioprotection) [25]. In clinical practice, this drug is used as a racemic mixture of 2 enantiomers, the R-(+)-enantiomer having also α1-receptor blocking activity [15]. The drug is highly lipophilic, exhibits rapid absorption and is subject to an extensive first-pass metabolism in the liver [15]. Cytochrome P450 (CYP) isoenzymes are involved in different path-ways of carvedilol's metabolism, of which isoenzyme CYP2D6 leads to formation of 5'-hydroxyphenyl carvedilol and 4'-hydroxyphenyl carvedilol, the last one being approximately 13 times more potent than carvedilol for the beta-blockade [29].

Sertraline is a potent and selective serotonin reuptake inhibitor, which is widely used for both antidepressant and antianxiety effects, in disorders like depression, obsessive-compulsive disorder, panic disorder, social phobia [12]. Sertraline is extensively metabolized by the liver *via* cytochrome P450, many isoforms of CYP (2B6, 2C9, 2C19, 2D6 and 3A4) being involved in converting sertraline into desmethyl-sertraline [16], an inactive *in vivo* metabolite [21]. *In vitro* [27] and *in vivo* studies [20, 28] have shown that sertraline mildly inhibits CYP2D6 and CYP3A4 isoenzymes' activity [14].

Due to carvedilol's extensive oxidative liver metabolism, drugs which induce or inhibit carvedilol's oxidation can affect its pharmacokinetics. As a 3rd generation β-blocker, carvedilol is well tolerated, even in the presence of comorbidities, and can be safely recommended for both young and elderly

patients [30]. Alongside paroxetine and escitalopram, sertraline is among the three most widely prescribed SSRIs [18]. Therefore, sertraline could be co-administered with carvedilol for the prevention or treatment of cardiovascular diseases associated with depression or with psychiatric disorders and consequently a pharmacokinetic drug-drug interaction between them should be taken into consideration. However, sertraline's effect on the pharmacokinetics of carvedilol *in vivo* has not been reported. Thus, the aim of this study was to evaluate whether a pharmacokinetic interaction occurs between carvedilol and sertraline in rats and to assess its magnitude. The results could have an impact on the safety profile and pharmacotherapy of carvedilol.

Materials and Methods

Chemicals and reagents

Carvedilol and sertraline were purchased from Moehs (Rubí, Spain). HPLC-grade acetonitrile, 98% formic acid and methanol of analytical-reagent grade were acquired from Merck KGaA (Darmstadt, Germany). Heparin sodium 25000 IU/5 mL (5000 IU/ mL) was purchased from Panpharma Laboratoires (France).

The equipment used in this study consisted of: BASi Culex ABC – Automatic Blood Collector (BASi, Indiana, USA), an Agilent 1100 series – HPLC system (binary pump, autosampler, thermostat) (Agilent Technologies, USA), coupled with a Bruker Ion Trap VL (Bruker Daltonics GmbH, Germany).

Animal treatment

In this preclinical study a group of 12 white male Wistar rats were included. Their weight was between 240 and 350 grams and the animals were purchased from the Experimental Animal Centre (Cluj-Napoca, Romania). The animal studies were performed after receiving approval from "Iuliu Haţieganu" University of Medicine and Pharmacy Ethics Committee.

The study was conducted in accordance with the specific regulations and amendments: the 43/2014 Law stating the protection of animals used for scientific research purpose published in "*Monitorul Oficial*" (Romania) and the "Guiding Principles in the Use of Animals in Toxicology" adopted by the Society of Toxicology (USA).

Before the preclinical trial, the rats were acclimated for several days by being kept in optimal conditions: clean room maintained at constant ambient temperature (22 ± 2°C) and humidity, under 12 h light - dark cycles. Each animal was placed in a solitary cage and was allowed free access to tap water *ad libitum* and specific rat diet.

Prior to being connected to BASi Culex ABC®, each rat had his left femoral vein cannulated in order to allow precise blood sampling at different time intervals after drug's administration. The surgery was performed under anaesthesia with a mixture of ketamine, xylazine and diazepam (1:1:1).

Study design

The study was designed as an open-label, sequential preclinical trial, that consisted of two periods. During the first period of study (Reference), each rat received an oral dose of 3.57 mg/kg body mass (b.m.) carvedilol, whilst during the second period (Test) 3.57 mg/kg b.m. carvedilol was orally co-administered with 7.14 mg/kg b.m. sertraline. Between the two periods, the rats were treated for 4 days with an oral dose of 7.14 mg/kg b.m. sertraline, given twice a day, in order to reach the steady-state concentration. Carvedilol (1 mg/mL) and sertraline (4 mg/mL) solutions for oral administration were prepared by using reference standards of pharmaceutical purity dissolved in saline solution and filtered through sterilizing membrane.

During both periods of study, a series of 200 µL venous blood samples were drawn into heparinized tubes after carvedilol administration at specified time intervals: 5, 10, 15, 20, 30, 45 minutes and 1, 2, 4, 8, 12, 18, 24, 30 hours after carvedilol administration.

Sample preparation

The sample preparation was carried out in Eppendorf tubes by the addition of 0.3 mL methanol over 0.1 mL blood. The tubes were sub-sequently vortex-mixed for 10 s and centrifuged for 5 minutes at 10000 rpm, 9167 g. The supernatant was afterwards transferred in an auto-sampler vial and 15 µL were injected into the chromatographic system.

High-performance liquid chromatography (HPLC) assay

Carvedilol plasma concentrations were estimated by a validated HPLC-MS method [1].

Pharmacokinetic analysis

The pharmacokinetic (PK) parameters of carvedilol when given alone or in combination with sertraline were determined by non-compartmental pharmaco-kinetic analysis. The calculation was performed by using the Phoenix WinNonlin 6.3 software (Pharsight Co., Mountain View, CA, USA). The maximum plasma concentration (C_{max}, ng/mL) and the corresponding time to reach the peak concentration (t_{max}, h) were obtained by visual inspection of individual plasma concentration *versus* time profiles. The area under the concentration-time curve from time zero to the last measurable concentration (AUC_{0-t}) was determined by using the linear trapezoidal rule. The area extrapolated to infinity ($AUC_{0-\infty}$) was calculated by adding C_t/k_{el} to AUC_{0-t}, where C_t is the last quantifiable plasma drug concentration and k_{el} is the first order elimination rate constant. The half-life time was further calculated as $0.693/k_{el}$.

Statistical analysis

The statistical analysis was performed by using Phoenix WinNonlin software version 6.3 (Pharsight Co.,

Mountain View, CA, USA). The data are presented as the mean value ± standard deviation (S.D.). The pharmacokinetic parameters from both study periods were compared using a one-way analysis of variance test (ANOVA) and a p value of < 0.05 was further considered for statistical significance. For t_{max}, the statistical significance between the two periods of study was evaluated using the nonparametric Friedman test.

Results and Discussion

The mean plasma concentration-time profiles of carvedilol following oral administration of carvedilol alone or in combination with sertraline, after a 4 days pre-treatment with the enzymatic inhibitor, are depicted in Figure 1.

The main pharmacokinetic (PK) parameters of carvedilol, when administered alone or with sertraline, as well as the statistical test results, are presented in Table I.

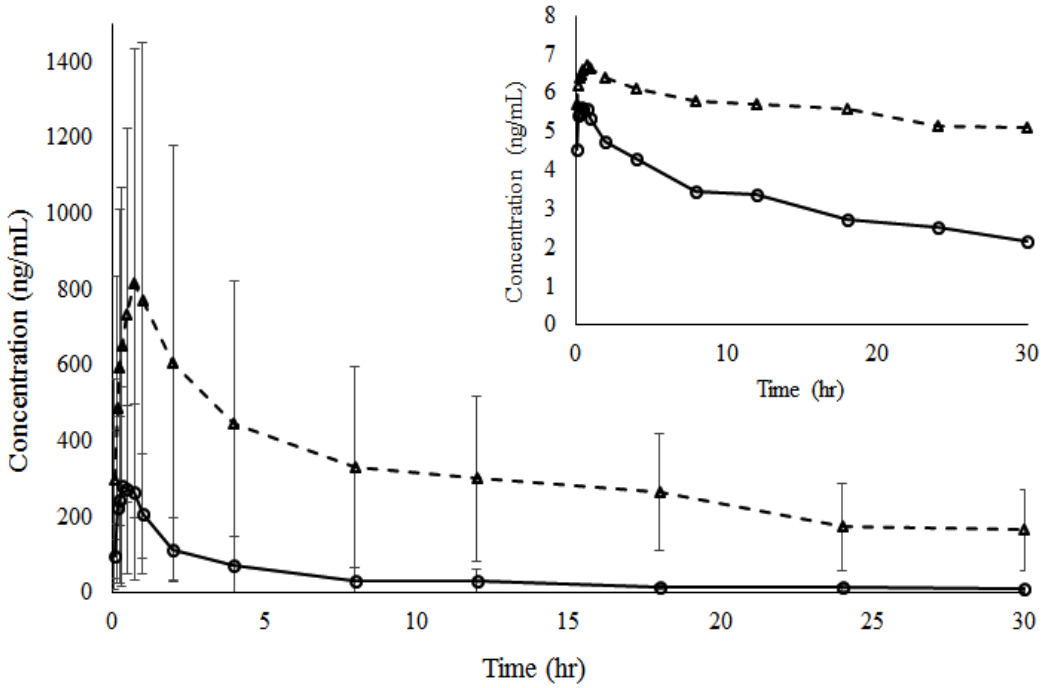

Figure 1.

Mean ± SD plasma concentrations of carvedilol, after oral administration of single dose carvedilol (3.57 mg/kg b.m.) alone (○) or in combination with sertraline (7.14 mg/kg b.m.) (Δ), after pre-treatment with sertraline for 4 days (n = 12). Inset: Semi-logarithmic presentation

Table I

Main pharmacokinetic (PK) parameters of carvedilol in rats (n = 12) after single oral dose of 3.57 mg/kg b.m. of carvedilol, before and after treatment with sertraline (7.14 mg/kg b.m.) for 4 days and the results of statistical test used for comparison

PK parameter (mean ± SD)	Carvedilol	Carvedilol+ Sertraline	p* value (ANOVA)
Cmax (ng/mL)	361.10 ± 260.07	903.78 ± 662.05	0.0082, S
tmax (hr)	2.20 ± 3.61	3.77 ± 7.41	Friedman, S
AUC0-∞ (ng*hr/mL)	1116.80 ± 634.50	8636.90 ± 4845.70	0.0000, S
kel (1/hr)	0.30 ± 0.42	0.06 ± 0.04	0.0021, S
t1/2 (hr)	5.37 ± 3.21	20.47 ± 20.52	0.0021, S
Cl_F (mL/hr/kg)	1054.29 ± 560.23	113.56 ± 78.48	0.0000, S
Vz_F (mL/kg)	7374.58 ± 6213.16	2564.57 ± 2254.56	0.0071, S

* statistically significant (S) when p < 0.05; Cmax - maximum (peak) plasma drug concentration; tmax – time to reach maximum (peak) plasma concentration following drug administration; AUC0-∞ – area under the plasma concentration - time curve from zero to infinity; kel – elimination rate constant from the central compartment; t1/2 – elimination half-life; Cl_F – apparent total clearance of the drug from plasma after oral administration; Vz_F – apparent volume of distribution during terminal after non-intravenous administration

The $AUC_{0-\infty}$ of carvedilol was considerably increased (by approximately 773.4%) in the presence of sertraline after oral administration of carvedilol (p < 0.05), therefore the total exposure over time to carvedilol was highly elevated. Moreover, the peak plasma concentration of carvedilol registered a 2.5 - fold increase during the test period, when co-administered with sertraline, and the time to reach

C_{max} was considerably delayed (statistically significant according to nonparametric Friedman test). The co-administration of sertraline led to an increase by 4.5 - fold of carvedilol's half-life time of elimination, due to the decrease of the k_{el}, all the observed differences between the two periods of study being statistically significant ($p < 0.05$). The decrease in body clearance (Cl_F) in case of sertraline co-administration is due to enzymatic inhibition of the main isoenzyme responsible for carvedilol's metabolism, CYP2D6 respectively.

The prevalence of depression among cardiac patients is acknowledged to be high [9]. Clinically significant depressive symptoms are present in 31 - 45% of patients with different cardiac diseases [9] and there are increasing evidences that support depression as a frequent comorbid condition in patients with heart impairment [24]. Moreover, depressed patients have a higher risk of developing hypertension [8]. Considering the clinical context and the fact that carvedilol is metabolized primarily *via* CYP2D6 isoenzyme [15] and sertraline is an inhibitor of this enzymatic metabolic pathway [17], it was of clinical importance to evaluate the magnitude of PK interaction between these two drugs.

Furthermore, since carvedilol is a P-gp substrate and sertraline is a P-gp inhibitor, the interaction between carvedilol and sertraline can be partially due to P-gp inhibition, but in a lower extent. The alteration of the main PK parameters like the elimination rate constant, body clearance and half-life are mainly associated with cytochrome P450 inhibition, meanwhile the increase in the area under the curve can be also due to P-gp inhibition.

The results of this preclinical trial prove the existence of a drug-drug interaction between carvedilol and sertraline of a high magnitude, results which are highly probable to have an impact on clinical practice. For instance, taking into consideration that the ergometric treatment responses are proportional with dose and both carvedilol enantiomers display linear pharmacokinetics [5], these results after a single oral dose are very likely to lead to major alterations in the pharmacokinetic profile of carvedilol after multiple dose treatment with this beta-blocker and therefore the risk of severe side effects should be taken into account. Furthermore, taking into consideration that the main isoenzyme responsible for carvedilol's metabolism (CYP2D6) exhibits polymorphism, which can further be influenced by the patients' ethnicity [23], gender and duration of cardiovascular impairment [19], the implications of these findings for poor metabolizers, in case of carvedilol-sertraline co-administration, should be a subject for therapeutic drug monitoring.

These findings are consistent with what had been previously reported for the safety profile of carvedilol. Other pharmacokinetic drug-drug interaction study showed interactions mediated by CYP2D6 isoenzyme inhibition in cases of carvedilol co-administered with fluoxetine [7] and paroxetine [22] which altered the PK parameters of carvedilol in a similar extent as that of sertraline. In both cases, CYP2D6 inhibition resulted in a significant increase in R(+) carvedilol area under the concentration time curve (by 77% for fluoxetine and by 78% for paroxetine) with a nonsignificant increase for the S(–) enantiomer [7, 22]. The pharmacokinetic interactions between carvedilol and antidepressants need to be studied due to their high magnitude and important clinical impact. Retrospective analysis of side effects in clinical trials showed that poor 2D6 metabolizers had a higher rate of dizziness during up-titration, presumably resulting from vasodilating effects of the higher concentrations of the α-blocking R(+) enantiomer [4].

In the recent years, the safety data of other β-blockers has been thoroughly evaluated. For instance, numerous clinical trials were conducted for nebivolol, a 3rd generation cardio-selective beta-blocker [2, 6], which proved the pharmacokinetic interactions between it and other antidepressants. Carvedilol and nebivolol are two 3rd generation beta-blockers of choice for heart failure [10]. On the contrary, the safety profile of carvedilol is less studied, the present study being one of the few to show the magnitude of interaction between carvedilol and an antidepressant, sertraline respectively, after concomitant use.

The use of the experimental rat model to evaluate this drug-drug interaction mediated primarily by CYP2D6, can bring some limitations, due to existing differences in enzyme activity between rat and human [3]. But considering the fact that the rat and human CYP2D isoforms share a high sequence identity (> 70%) and that among these isoforms, CYP2D1 is the rat orthologue of the human CYP2D6 [13, 26], the animal model can be used to predict the metabolic behaviour of carvedilol in humans. Nevertheless, additional clinical trials on healthy volunteers and hypertensive patients should be conducted in order to decide the clinical relevance of this proved interaction.

Conclusions

The aim of this study was to provide data on carvedilol safety in concomitant use with sertraline. The pharmacokinetic profile after a single oral dose of carvedilol was statistically significant altered by the multiple-dose pre-treatment with sertraline, a p value < 0.05 (ANOVA) being found for all the evaluated PK parameters between the two periods of the study. The increased exposure to carvedilol is due to enzymatic inhibition via CYP2D6 and may have further implications for clinical therapeutics.

Under the restriction that results were obtained in rats and steady-state concentrations were present

only for sertraline, a meaningful pharmacokinetic interaction between carvedilol and sertraline could not be proven. Until further investigations decide the clinical consequences of a long-term therapy with carvedilol and sertraline, caution should be considered when prescribing this combination in clinical practice and safety monitoring of this combination should be recommended.

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