Influence of fluvoxamine on carvedilol metabolism and plasma disposition – *in vitro* and *in vivo* experiments

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Graphical Abstract

FVX-CVD - *in vitro* and *in vivo* interactions
Abstract: Carvedilol is one of the most used cardiovascular drugs, highly metabolized by CYP450 2D6, 1A2, 2C9. Fluvoxamine, an antidepressant agent, is a moderate/potent inhibitor of these enzymes. There is the risk of drug-drug interaction when these two drugs are concomitantly administered. The aim of this study was to investigate the drug-drug interactions between carvedilol and fluvoxamine in vitro and in rats.

There were two periods: reference and test. In the first period, each rat received an oral dose of 3.57 mg/kg body weight [b.w.] carvedilol). In the test period, carvedilol was administered after a pre-treatment with multiple oral doses of fluvoxamine (14.28 mg/kg b.w.). HPLC-MS was the device used to determine the plasma concentration of carvedilol. The PK parameters were calculated by noncompartmental analysis. Rat liver microsomal incubation systems were used to investigate the effect of fluvoxamine on the metabolic rate of carvedilol.

Fluvoxamine co-administered with carvedilol changed the PK parameters (increase AUC, t1/2, decrease the Cl). The in vitro experiment showed that fluvoxamine decrease the metabolic rate of carvedilol.

The present study demonstrated the pharmacokinetic drug-drug interaction between carvedilol and fluvoxamine in vitro and in vivo. Fluvoxamine significantly influenced the pharmacokinetic of carvedilol, due to its capacity of CYP2D6 and CYP1A2 inhibition. As a result of this interaction the exposure to carvedilol was significantly increased. This is the reason why co-administration of carvedilol and fluvoxamine needs precaution.

Keywords: Carvedilol  Fluvoxamine  Cytochrome P450  Enzyme inhibitor
Introduction

- **Carvedilol** is a nonselective β-blocking agent, which is commonly used for the treatment of different cardiovascular diseases like hypertension, cardiac insufficiency, left ventricular dysfunction developed after a myocardial infarction.

- Carvedilol is metabolized via cytochrome P450, CYP2D6, CYP1A2 and CYP2C9 isoforms and by glucuronidation.

- **Fluvoxamine** is an antidepressant known to have inhibitory effect on cytochrome P450 (CYP2D6 and CYP1A2).

- The aim of this study was to investigate whether a pharmacokinetic interaction occurs between carvedilol and fluvoxamine in rats; the results may have an impact on the safety pharmacotherapy with carvedilol.
Materials and methods

Subjects: 13 white male Crl:WI rats were included in the study, with a medium body-weight (b.w.) 330±20 g. The working protocol was reviewed and approved by the Ethics Committee of the University of Medicine and Pharmacy “Iuliu Hațieganu”, Cluj-Napoca, Romania.

In vivo experimental design
Each rat was cannulated on the femoral vein and then connected to BASi Culex ABC®.

2 periods: Reference (I) and Test (II)
- Period I: 3.57 mg/kg b.w. carvedilol (orally)
- Inhibitor pretreatment: 14.28 mg/kg b.w. fluvoxamine (orally), 4 days
- Period II: 3.57 mg/kg b.w. carvedilol + 14.28 mg/kg b.w. fluvoxamine

200 µL venous blood was drawn during both periods of study, at 5, 10, 15, 20, 30, 45 min. and 1, 2, 4, 8, 12, 18, 24, 30 hr after the carvedilol administration; the samples were stored frozen (-20°C) until the analysis (by validated HPLC-MS method).
Materials and methods

In vitro experimental design
- Rat liver microsomes were isolated by differential centrifugation. The microsomal incubations were performed at 37°C with 0.5 and 1 μM carvedilol and 0, 0.1, 0.75, 1.5 μM citalopram, with 0.25 mg microsomal protein/mL. The reaction was initiated by adding the NADPH regenerating system and was terminated by cooling on ice and adding cold acetonitrile at different time points (0.5, 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210 and 240 minutes). Concentrations were thereby determined by HPLC-FLD assay. The incubations were completed in triplicate.

Pharmacokinetic analysis
- Non-compartmental pharmacokinetic analysis – PK parameters of carvedilol: $C_{\text{max}}$ (ng/mL), $t_{\text{max}}$ (hr), $AUC$ (ng*hr/mL), $k_{\text{el}}$ (1/hr), $t_{1/2}$ (hr), $Cl_F$ (mL/hr/kg), $Vz_F$ (mL/kg).

Statistical analysis
- ANOVA was used for intergroup comparison, $p<0.05$ for all analyses
Results and discussion

Figure 1. Mean ± SD plasma concentrations of carvedilol, after oral administration of single dose carvedilol (3.57 mg/kg b.w.) alone (Δ) or in combination with citalopram (1.42 mg/kg b.w.) (○), after pre-treatment with fluvoxamine for 3 days (n=13). Inset: Semi-logarithmic presentation.
Results and discussion

Table 1. Main pharmacokinetic (PK) parameters of carvedilol in rats (n=13) after single oral dose of 3.57 mg/kg b.w. of carvedilol, before and after treatment with fluvoxamine (14.28 mg/kg b.w.) for 3 days and their statistical comparison (statistically significant (S) when \( p<0.05 \))

<table>
<thead>
<tr>
<th>PK parameter (mean ± SD)</th>
<th>CARV</th>
<th>CARV + CIT</th>
<th>p value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (ng/mL)</td>
<td>361.10±260.07</td>
<td>528.50±288.20</td>
<td>0.0651, NS</td>
</tr>
<tr>
<td>( t_{\text{max}} ) (hr)</td>
<td>2.12±3.62</td>
<td>0.95±1.31</td>
<td>Kruskal-Wallis, NS</td>
</tr>
<tr>
<td>AUC(ng*hr/mL)</td>
<td>1113.42±661.97</td>
<td>2299.96±1465.32</td>
<td>0.0059, S</td>
</tr>
<tr>
<td>( k_{\text{el}} ) (1/hr)</td>
<td>0.30±0.42</td>
<td>0.09±0.05</td>
<td>0.0188, S</td>
</tr>
<tr>
<td>( t_{1/2} ) (hr)</td>
<td>5.29±3.22</td>
<td>12.42±12.69</td>
<td>0.0188, S</td>
</tr>
<tr>
<td>( Cl_F ) (L/hr/kg)</td>
<td>3.68±2.13</td>
<td>2.03±1.39</td>
<td>0.0174, S</td>
</tr>
<tr>
<td>( Vz_F ) (L/kg)</td>
<td>25.03±21.29</td>
<td>27.49±19.94</td>
<td>0.5858, NS</td>
</tr>
</tbody>
</table>

*statistically significant (S) when \( p < 0.05 \)
Results and discussion

Table 2. The percentage of carvedilol metabolized and the percentage of metabolite, 4’-hydroxyphenyl carvedilol resulted for the in vitro experiment, after 30 min of incubation in rat-pooled liver microsomes systems (mean ± SD)

<table>
<thead>
<tr>
<th>Substrate concentration, μmol/L</th>
<th>0.5</th>
<th>1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibitor concentration, μmol/L</td>
<td>CVD</td>
<td>4’-OH CVD</td>
</tr>
<tr>
<td>0</td>
<td>99.05±0.63</td>
<td>23.81±3.7</td>
</tr>
<tr>
<td>0.1</td>
<td>90.44±1.28</td>
<td>17.56±0.42</td>
</tr>
<tr>
<td>0.75</td>
<td>78.48±4.16</td>
<td>14.86±1.82</td>
</tr>
<tr>
<td>1.5</td>
<td>71.73±0.42</td>
<td>12.63±0.57</td>
</tr>
</tbody>
</table>

CVD, carvedilol; 4’-OH CVD, 4’-hydroxyphenyl carvedilol
Results and discussion

Figure 2. Rate of metabolism ± SD of carvedilol in relation to concentration of inhibitor (fluvoxamine) in rat liver microsomal preparations. Incubations were performed for 30 minutes in the control state (no inhibitor), and with varying concentrations of citalopram for 0.5 μM carvedilol (•) and 1 μM carvedilol (▲).
Results and discussion

Figure 3. AUC$_{0-30}$ ± SD of carvedilol in relation to the concentration of the inhibitor (fluvoxamine) in rat-pooled liver microsomal preparations. Incubations were performed in the control state (no inhibitor) and with varying concentrations of citalopram for 0.5 μM carvedilol (•) and 1 μM carvedilol (●) (n = 3).
Results and discussion

- The results of this preclinical trial and the *in vitro* experiments prove the existence of a *metabolic drug-drug* interaction between *carvedilol* and *fluvoxamine*; this can have an impact on clinical practice for those patients who follow a treatment with carvedilol and fluvoxamine.

- An *elevated exposure* over time to carvedilol was indicated by *statistically significant* alteration ($p<0.05$) of pharmacokinetic parameters ($\text{AUC}_{0-\infty} \uparrow 2.06 \times, k_{el} \downarrow 3.33 \times, t_{1/2} \uparrow 2.34 \times, \text{Cl} \downarrow 0.55 \times$) after fluvoxamine pretreatment.

- The *alteration of carvedilol’s metabolism* appeared as a result of this drug-drug interaction, fluvoxamine inhibits the main isoenzymes (CYP2D6, CYP1A2) which are involved in metabolization of carvedilol.

- Many preclinical and clinical previous studies demonstrated the pharmacokinetic drug-drug interaction between carvedilol and other CYP450 inhibitors, including citalopram, bupropion, fluoxetine, paroxetine, ketoconazole and voriconazole; the results were similar with that obtained by fluvoxamine inhibition in the present study.
Conclusions

- The results demonstrated that co-treatment with fluvoxamine influences the pharmacokinetics of a single oral dose of carvedilol.

- Fluvoxamine co-administration led to a significant alteration of carvedilol’s pharmacokinetic profile in rats, also demonstrated in vitro, these effects could be explained by the existence of a drug-drug interaction mediated by CYP2D6 and CYP1A2 inhibition.

- A definite conclusion about the clinical significance of this drug interaction cannot be reached without performing other similar studies which include patients.
Acknowledgments

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