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

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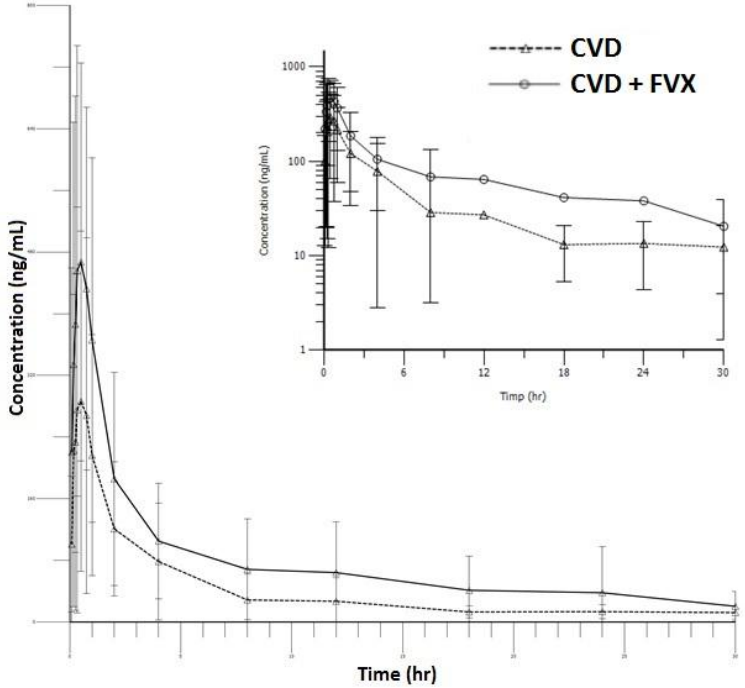
Graphical Abstract

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, $t_{1/2}$, 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 Haţieganu", 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: $\mathbf{C_{max}}$ (ng/mL), $\mathbf{t_{max}}$ (hr), **AUC** (ng*hr/mL), $\mathbf{k_{el}}$ (1/hr), $\mathbf{t_{1/2}}$ (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

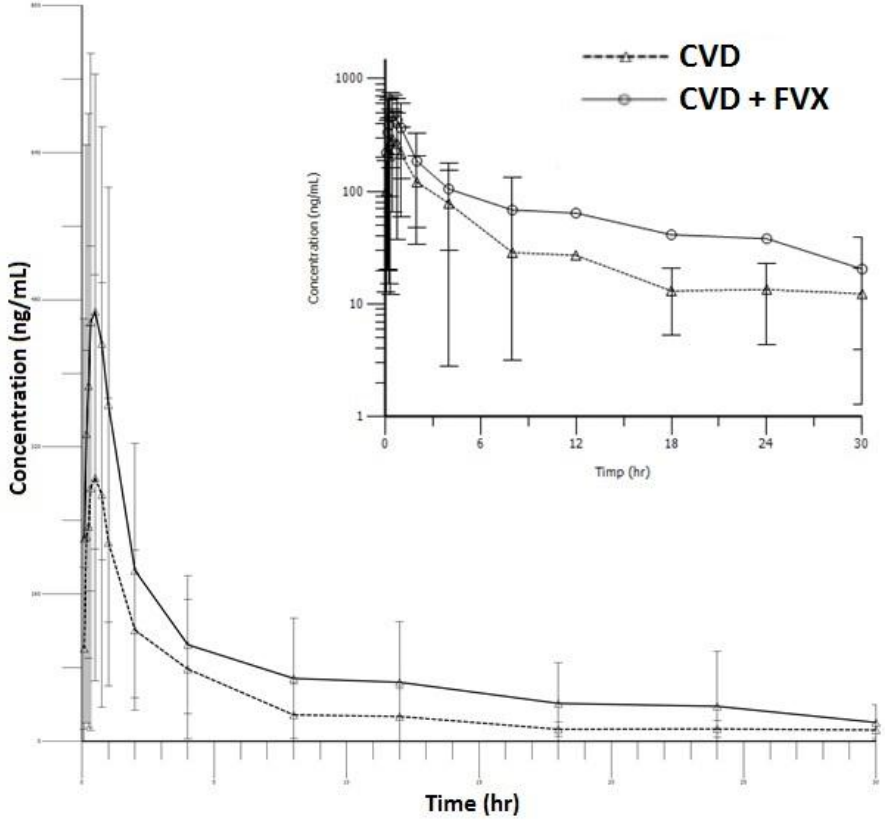


Figure 1. Mean ± SD **plasma concentrations** of **carvedilol**, after oral administration of single dose carvedilol (3.57 mg/kg b.w.) alone (Δ) or in combination with **citalopram** (1.42 mg/kg b.w.) (○), after pre-treatment with fluvoxamine for 3 days (n=13). Inset: Semi-logarithmic presentation

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**

PK parameter (mean ± SD)	CARV	CARV + CIT	p value (ANOVA)
C_{max} (ng/mL)	361.10±260.07	528.50±288.20	0.0651, NS
t_{max} (hr)	2.12±3.62	0.95±1.31	Kruskal-Wallis, NS
AUC(ng*hr/mL)	1113.42±661.97	2299.96±1465.32	0.0059, **S**
k_{el} (1/hr)	0.30±0.42	0.09±0.05	0.0188, **S**
$t_{1/2}$ (hr)	5.29±3.22	12.42±12.69	0.0188, **S**
Cl_F (L/hr/kg)	3.68±2.13	2.03±1.39	0.0174, **S**
Vz_F (L/kg)	25.031±21.29	27.49±19.94	0.5858, NS

*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)

Substrate concentration, µmol/L	0.5		1	
Inhibitor concentration, µmol/L	CVD	4'-OH CVD	CVD	4'-OH CVD
0	99.05±0.63	23.81±3.7	94.46±4.2	16.77±1.22
0.1	90.44±1.28	17.56±0.42	85.08±1.96	13.65±1.58
0.75	78.48±4.16	14.86±1.82	74.6±2.32	10.4±0.38
1.5	71.73±0.42	12.63±0.57	70.64±0.71	9.71±0.35

CVD, carvedilol; 4'-OH CVD, 4'-hydroxyphenyl carvedilol

Results and discussion

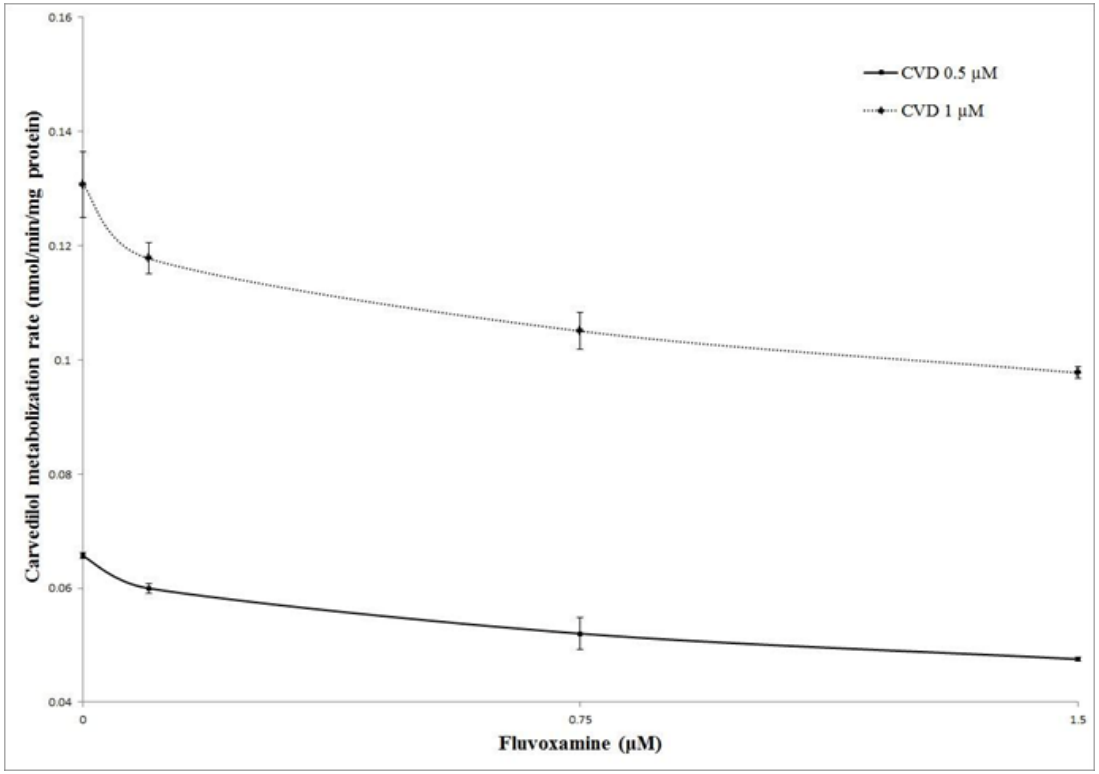


Figure 2. Rate of metabolism ± SD of **carvedilol** in relation to concentration of **inhibitor (fluvoxamine)** in rat liver microsomal preparations. Incubations were performed for 30 minutes in the control state (no inhibitor), and with varying concentrations of citalopram for 0,5 µM carvedilol (•) and 1 µM carvedilol (▲).

Results and discussion

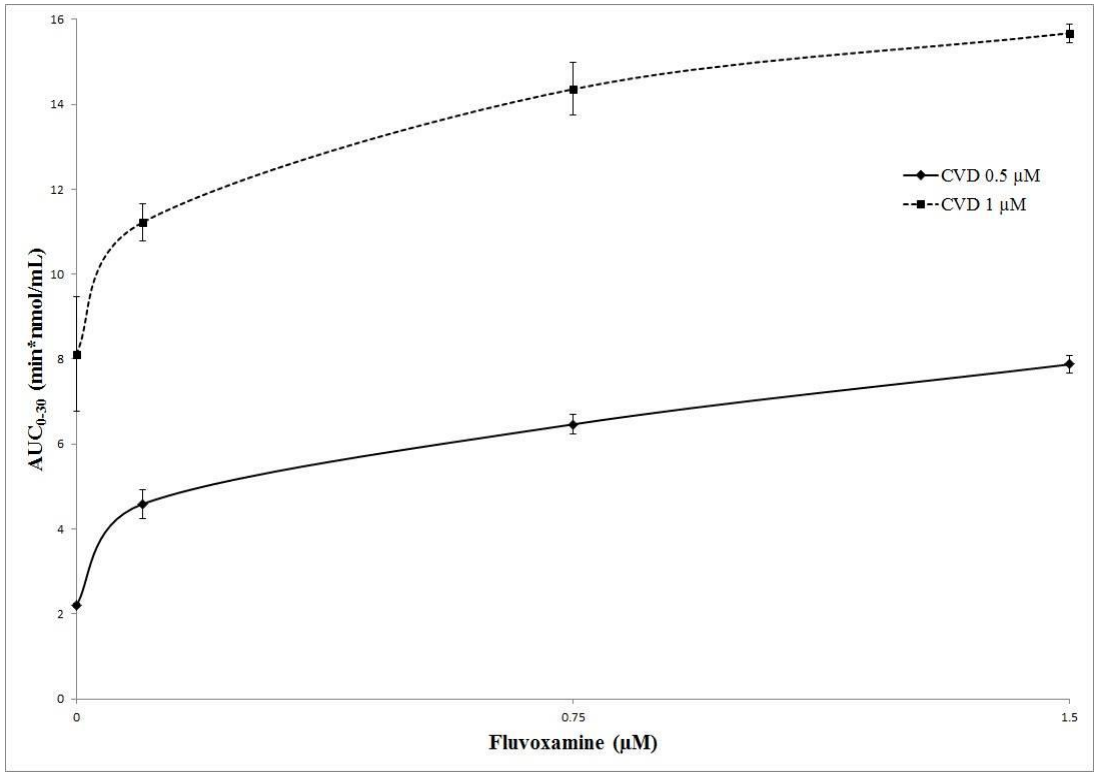


Figure 3. $AUC_{0–30}$ ± SD of **carvedilol** in relation to the concentration of the **inhibitor (fluvoxamine)** in rat-pooled liver microsomal preparations. Incubations were performed in the control state (no inhibitor) and with varying concentrations of citalopram for 0,5 µM carvedilol (•) and 1 µM 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 ($AUC_{0-\infty}$↗2.06 x, k_{el}↘3.33 x, $t_{1/2}$↗2.34 x, Cl↘0.55x) after fluvoxamine pretreatment
- The **alteration of carvedilol's metabolism** appeared as a result of this drug-drug interaction, fluvoxamine inhibits the main izoenzymes (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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