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Influence of Amiodarone on Urinary Excretion of 6β-Hydroxycortisol in Humans

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Summary

The present study was undertaken to evaluate the use of cortisol 6β-hydroxylation in defining the effect of amiodarone on cytochrome CYP3A activity. To accomplish this goal, the *in vivo* activity of CYP3A was estimated by measuring the 24-hour urinary excretion of 6β-hydroxycortisol (6β-OHC) and by calculating 24-hour ratio of 6β-hydroxycortisol to urinary free cortisol (6β-OHC/UFC ratio). Nine cardiac patients scheduled for amiodarone treatment were recruited to participate in this study. Urine was collected over a 24-hour period from each subject before the first amiodarone administration and during the third day of oral administration of amiodarone (200 mg four times daily as a loading dose). Three days of amiodarone treatment caused a significant decrease ($p<0.05$) in both the 6β-OHC/UFC ratio and the 24-hour urinary excretion of 6β-OHC. These results suggest that amiodarone is an inhibitor of CYP3A activity.

Key words
Amiodarone • Cortisol • 6β-hydroxycortisol • Cytochrome CYP3A

Introduction

Amiodarone is the most effective agent in the control of ventricular tachycardia and fibrillation, with an efficacy comparable to that of implantable cardioverter-defibrillators (Singh 1996). The pharmacokinetics of this agent differ from those of any other antiarrhythmic drug currently available. Amiodarone has a large volume of distribution (5000 l) and a long elimination half-life (20-60 days) (Freedman and Somberg 1991). Because of its very limited water solubility, it has to undergo extensive hepatic metabolism prior to final elimination into bile (Roden 1993). Although several metabolites are formed during this process, only N-desethylamiodarone (DEA) has been detected in humans (Flanagan *et al.* 1982). Fabre *et al.* (1993) have shown that conversion of amiodarone to its major metabolite DEA is catalyzed by the enzymes of cytochrome CYP3A subfamily. Unfortunately, the large lipophilic molecule of amiodarone is not only attracted to the cytochrome CYP3A for its metabolism, but this drug also competitively inhibits the activity of these enzymes (Funck-Brentano *et al.* 1994). As a consequence, drug interactions with amiodarone at CYP3A become a significant issue in the treatment of arrhythmias in cardiac patients because numerous drugs applied in the treatment and prophylaxis of concomitant diseases (e.g. calcium channel antagonists, terfenadine, midazolam,

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itraconazole or ketoconazole) utilize CYP3A for metabolism. Actually, several *in vitro* studies have described the inhibitory effect of amiodarone and its major metabolite DEA on cytochrome CYP3A metabolism (Fabre *et al.* 1993, Trivier *et al.* 1993). In contrast, much less information is available concerning *in vivo* observations (Chitwood *et al.* 1993, Ha *et al.* 1996).

Several probe-based methods have been proposed for measuring CYP3A activity *in vivo*, including the erythromycin breath test, midazolam clearance after oral administration or measurement of 6β-hydroxycortisol (6β-OHC) excretion in the urine. Among these methods, measurement of 6β-OHC is the only truly non-invasive assay of CYP3A activity. This makes it an interesting method for clinical settings. 6β-hydroxycortisol, a hydrophilic metabolite formed *via* hydroxylation by CYP3A, is the minor unconjugated urinary product of cortisol accounting for approximately 1 % of total daily cortisol secretion (Canalis *et al.* 1982). Urinary 6β-hydroxycortisol excretion has been proved to be a specific marker of CYP3A induction (Kovacs *et al.* 1998). There appears to be a significant positive correlation between the urinary 6β-OHC level and both liver microsomal cortisol 6β-hydroxylase activity and the CYP3A liver content (Ged *et al.* 1989). Changes due to circadian variations in cortisol production can be corrected either by using 24-hour urine collection or by expressing the results as a ratio of 6β-hydroxycortisol to urinary free cortisol (6β-OHC/UFC ratio) (Saenger 1983, Ohnhaus *et al.* 1989).

The aim of the present study was to evaluate the influence of orally administered amiodarone on CYP3A activity. To accomplish this goal, we utilized the 24-hour urinary 6β-hydroxycortisol excretion and the 24-hour 6β-OHC/UFC ratio as *in vivo* probes for CYP3A activity.

Methods

The protocol of the study was approved by the Human Ethics Committee of the Charles University Teaching Hospital in Hradec Králové. All subjects participating in the study gave their signed written informed consent. The study was conducted at the Second Department of Internal Medicine in Hradec Králové.

Reagents and solutions

6β-hydroxycortisol and 6β-hydroxycortisone were purchased from Sigma Co. (St. Louis, MO, USA). The RIA immunoassay test kit for urinary free cortisol was obtained from Immunotech (Prague, Cat. No. 1841). All other chemicals and solvents were of the highest analytical-reagent grade commercially available.

Table 1. Clinical characteristics of the patients.

Patient No.	Age (years)	Diagnosis	Concomitant drugs
1.	51	Ischemic heart disease	metoprolol, isosorbide dinitrate, acetylosalicylic acid, hydrochlothiazide
2.	75	Ischemic heart disease	molsidomine, isosorbide dinitrate, acetylosalicylic acid, heparin
3.	67	Ischemic heart disease	metoprolol, isosorbide dinitrate, acetylosalicylic acid
4.	62	Ischemic heart disease	carvedilol, furosemide, molsidomine, acetylosalicylic acid, insulin
5.	54	Ischemic heart disease	isosorbide dinitrate, acetylosalicylic acid, molsidomine, amiloride
6.	61	Ischemic heart disease	isosorbide dinitrate, molsidomine, carvedilol, acetylosalicylic acid, ticlopidine
7.	41	Cardiomyopathy	carvedilol, acetylosalicylic acid
8.	64	Ischemic heart disease	isosorbide dinitrate, molsidomine
9.	74	Ischemic heart disease	isosorbide dinitrate, acetylosalicylic acid, hydrochlothiazide, heparin

Subjects

The studied subjects were patients who were to be treated with amiodarone for ventricular tachycardia or ventricular fibrillations that was reproducibly detected during a baseline electrophysiologic study. Nine consecutive consenting men aged from 41 to 75 years (median = 62) were enrolled. The clinical characteristics of these patients are presented in Table 1. None of the 9 patients was receiving medication known or suspected to induce or inhibit the catalytic activity of CYP3A (glucocorticoids, antiepileptic drugs, imidazole antimycotic drugs or macrolide antibiotics).

Study design

This was an open-label study in which each patient was subjected to a 4-day observation period. During this period, patients were hospitalized at the Department of Internal Medicine. Basic electrophysiological recordings were performed. Exactly at 06:00 h on the following morning, patients emptied their bladders. Urine was then collected over a 24-hour period until the next morning, when patients started amiodarone treatment with a loading dose of 800 mg/day (four tablets of 200 mg Cordarone, Sanofi Winthrop Idustrie, Ambares, France) for 14 days. Second 24-hour urine samples were obtained during the third day of amiodarone therapy at the same time interval (from 6:00 to 6:00 h next day). All of the urine samples were collected without preservatives and stored at –20 °C until assayed.

Measurement of CYP3A activity

The activity of CYP3A was estimated by calculating the ratios of 6β-hydroxycortisol to urinary free cortisol (6β-OHC/UFC) concentrations in 24-hour urine samples and by measuring the 24-hour urinary excretion of 6β-hydroxycortisol.

Analytical methods

The concentrations of 6β-hydroxycortisol in the urine were measured by the HPLC method described by Ono *et al.* (1986). The within-run and between-run variations of this method were below 8 %. The CORTISOL RIA kit was used for the determination of free cortisol in urine samples (directly, without extraction). The intra-assay variation was 2.8-5.8 % and the inter-assay variation was 5.4-9.2 %.

Data analysis

The *in vivo* results obtained before and during amiodarone administration were compared by the Wilcoxon signed-rank test using NCSS computer software (release 6.0.21 of NCSS 6.0, Kaysville, Utah). A difference was considered statistically significant if the probability of erroneously rejecting the null hypothesis of no difference was less than 5 %.

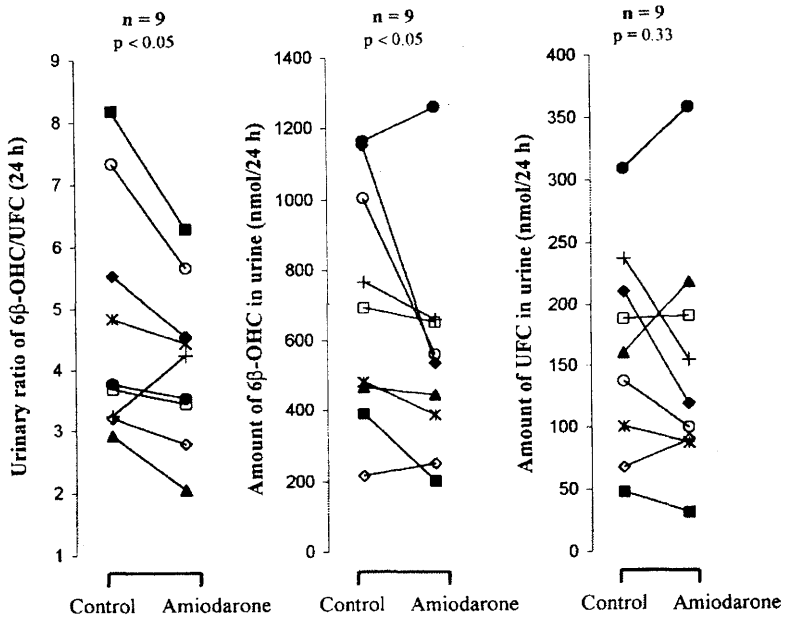

Fig. 1. *Metabolic ratio of 6β-hydroxycortisol to urinary free cortisol (left panel), 24-hour urinary excretion of 6β-hydroxycortisol (middle panel) and free cortisol (right panel) before (Control) and during (Amiodarone) amiodarone administration. Each patient has particular symbol.*

Results

Amiodarone was well tolerated in all patients. None of the subjects withdrew from the study. Individual results for the ratio of 6β-hydroxycortisol to urinary free cortisol before and during amiodarone administration are shown in Figure 1 (left panel). Eight out of nine patients had decreased 6β-OHC/UFC ratio during the third day of treatment (loading dose 800 mg/day), whereas one patient showed an increase. The overall inhibitory effect of AM on the urinary ratio of 6β-OHC/UFC was statistically significant ($p<0.05$).

The predose and withindose values of the 24-hour urinary excretion of 6β-hydroxycortisol are shown in Figure 1 (middle panel). During the third day of amiodarone therapy, 7 out of 9 patients had decreased 24-hour urinary excretion of 6β-hydroxycortisol as compared with the control levels, while increases were

observed in two patients (Fig. 1, middle panel). The overall inhibitory effect of amiodarone on 24-hour urinary excretion of 6β-hydroxycortisol was significant ($p<0.05$). Changes in the total daily excretion of 6β-hydroxycortisol were not associated with changes in the 6β-OHC/UFC ratio; i.e. the two patients with increased excretion had decreased 6β-OHC/UFC ratio and, conversely, one patient with an increased ratio had a decreased excretion of 6β-hydroxycortisol. Patients failed to exhibit any consistent change in the 24-hour urinary excretion of free cortisol (Fig. 1, right panel).

Discussion

This study was aimed at evaluating the potential influence of amiodarone therapy on CYP3A activity. To assess CYP3A activity we prospectively used cortisol 6β-hydroxylation as a marker reaction. The reason for this choice was the fact that amiodarone is reserved only for patients with serious tachyarrhythmias (because of a wide spectrum of adverse effects), who should be treated as soon as possible. Therefore, complicated invasive methods are inappropriate.

It is generally accepted that the measurement of urinary excretion of 6β-hydroxycortisol and free cortisol is useful in the detection of enzyme-inducing effects of drugs on CYP3A. This approach has been successfully employed for the assessment of CYP3A induction by phenobarbitone (Ohnhaus *et al.* 1989), rifampicin (Kovacs *et al.* 1998), rifampin (Tran *et al.* 1999), phenytoin (Fleishaker *et al.* 1995) and carbamazepine (Tomlinson *et al.* 1996). In contrast to these findings, different results have been reported concerning the utilization of cortisol 6β-hydroxylation as a marker of CYP3A inhibition. It has, for example, been shown that cortisol 6β-hydroxylation along with dapsone N-hydroxylation failed to show any consistent effect of three protease inhibitors (ritonavir, indinavir, amprenavir) on CYP3A activity. This is interesting, especially for ritonavir, since this drug appears to be one of the most potent inhibitors of CYP3A (Gass *et al.* 1998). In another study, the ratio of 6β-hydroxycortisol to cortisol was significantly decreased in the 0-4 h fraction of urine after ingestion of grapefruit juice, but not in the 4-24 h fraction or for compiled data (fraction 0-24 h) (Seidegard *et al.* 1998). On the other hand, several papers have clearly demonstrated the usefulness of this assay for evaluating CYP3A inhibition. For example, Tran *et al.* (1997) have shown that inhibition of CYP3A by stiripentol was followed by both a slowing of dextromethorphan N-demethylation and a decrease in the 6β-OHC/UFC ratio. There was no significant difference between the results of these two tests. Furthermore, the ratio of 6β-hydroxycortisol to cortisol in the urine decreased to 50 % of the original level during treatment of volunteers with 200 mg/day of fluconazole (Morita *et al.* 1992). At this dose, fluconazole effectively inhibited CYP3A as demonstrated by an increase in plasma concentrations of orally administered cyclosporine (a twofold increase in plasma AUC and trough concentrations) (Canafax *et al.* 1990) and midazolam (3.5fold increase in plasma AUC) (Olkkola *et al.* 1996).

In our study, changes in the 24-hour excretion of 6β-hydroxycortisol and in the ratio of 6β-hydroxycortisol to urinary free cortisol led to similar observations. The medians of both above mentioned parameters were considerably decreased after a 3-day treatment with amiodarone indicating that amiodarone therapy lowered CYP3A activity. This rapid onset of the inhibitory action of amiodarone is most probably caused by rapid distribution of this agent from the central compartment into tissue compartments ($t_{0.5\alpha}$ = 4.6-18.7 hours) (Freedman and Somberg 1991). Brennan *et al.* (1991) demonstrated that during the fourth day of amiodarone therapy with a daily dose of 800 mg, trough plasma concentrations of amiodarone and N-desethylamiodarone (blood samples were obtained immediately prior to the morning dose) were 0.49±0.18 and 0.18±0.04 mg/l (mean±S.D.) respectively. The results in this study showed that plasma concentrations in the steady state in patients on a maintenance dose of 400 mg/day for five days a week are approximately 3-times (AM) and 6-times (DEA) higher. Thus, the extent of CYP3A inhibition in the steady state can be much higher than that observed in our study.

In conclusion, the results of our study show that the activity of CYP3A, as measured by cortisol 6β-hydroxylation, is reduced even in the early phase of amiodarone therapy. Therefore, the dosage of drugs metabolized by enzymes of the CYP3A subfamily may need to be adjusted when coadministered with amiodarone.

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