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IMPACT OF AMPHOTERICIN B ON THE CYTOCHROME P450 SYSTEM IN HIV-INFECTED PATIENTS

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Abstract
Objective: To investigate whether cytochrome P450-dependent enzymes are influenced by amphotericin B (Am-B) during the treatment of Candida oesophagitis in HIV-infected patients.
Methods: Twelve HIV-infected, antiretroviral-naive patients (CDC/WHO stage C3) with Candida oesophagitits were enrolled into a prospective clinical trial. The patients were treated with Am-B (0.4 mg/kg body weight) for two weeks. At baseline and after Am-B therapy the clearance of antipyrine and its metabolites were investigated by high-performance liquid chromatography. In addition, the urinary excretion of 6-β-hydroxycortisol and 17-hydroxycorticosteroids was assessed by means of a radioimmunoassay.
Results: The following significant changes were observed after Am-B treatment ($P < 0.01$): increase of antipyrine half-life (12.4 h vs 16.8 h) and the area under the plasma concentration-time curve (27.9 mg min/ml vs 38.1 mg min/ml); decrease of the total body clearance (61.2 ml/min vs 43.7 ml/min); decrease of the renal clearance of antipyrine metabolites - norantipyrine (7.45 ml/min vs 5.31 ml/min), 4-hydroxyantipyrine (15.4 ml/min vs 10.3 ml/min), hydroxymethylantipyrine (4.31 ml/min vs 3.65 ml/min); decrease of urinary 6-β-hydroxycortisol excretion (453 μg/24h vs 298 μg/24h) and the ratio of 6-β-hydroycortisol to 17-hydroxycorticosteroids (8.8% vs 6.4%).
Conclusions: Our data indicate that Am-B therapy has an inhibitory effect on cytochrome P450-dependet enzymes in HIV-infected patients. These results are of particular significance for HIV-infected patients who are concomitantly treated with drugs that are predominantly metabolised in the liver. A careful drug monitoring system seems advisable, especially for proteinase inhibitor experienced HIV-1-infected subjects.

Key words: Amphotericin B; cytochrome P450; HIV

INTRODUCTION

More than 90% of patients with AIDS develop oropharyngeal candidiasis. Candida oesophagitis is also of concern, since it occurs in more than 10% of patients with AIDS. The azoles, both fluconazole and itraconazole, are an integral part of the management of Candida oesophagitis. In patients with azole-resistant Candida oesophagitis or cryptococcal menigitis, treatment with the polyene macrolide amphothericin B (Am-B) is recommended. Amphothericin B is considered the most effective antifungal agent used for the management of systemic mycoses in humans. Because of its pronounced adverse effects such as severe nephrotoxicity, the clinical use of systemic Am-B is limited, however [1, 2].

The efficacy of Am-B is probably due to two interrelated effects: 1) an increase of permeation by binding to sterols in cellular membranes 2) a prooxidant effect causing oxidative damage in fungal target cells [3, 4]. However, these effects may potentially alter cellular membrane functions, resulting in organ dysfunction. The highest Am-B concentrations are reached in the liver with the potential to alter hepatic cellular integrity. Previously it has been demonstrated that Am-B reduces bile flow and decreases bile acid secretion of perfused rat livers [5]. In animal experiments, it has been shown that Am-B reduces the concentration of the hepatic microsomal cytochrome P450 and results in a decrease of antipyrine clearance [6-8], which is a common biomarker for Cytochrome P-450 activity. However, (to the best of our knowledge) sparse data are available about effects of Am-B on the hepatic cytochrome P450 system in humans. This issue is of great significance for HIV-infected patients concomitantly receiving proteinase inhibitors and/or other drugs that undergo hepatic metabolism. Protease inhibitors have been shown to decrease antipyrine metabolism in rats [9].The rationale of the present study was to investigate whether or not Am-B might have effects on the cytochrome P450 system in HIV-infected patients. During the treatment with AM-B we therefore assessed the metabolic capacity and function of monooxygenases as well as the excretion of 6-β-hydroxycortisol and 17-hydroxycorticosteroids. Endogenous cortisol metabolism represents a marker for CYP 3A4 metabolism, which acts as a major enzyme involved in the metabolism of protease inhibitors [10].

Patients and Methods

The study was approved by the university's ethical committee, and all patients were informed about the study and gave written consent before participation. In a prospective trial we recruited 12 non-smoking antiretroviral-naive HIV-positive patients (CDC/WHO stage C3) suffering from Candida oesophagitis. Patients who suffered from any other new-onset infection were excluded from the study. No evidence for hepatic or renal insufficiency was present in any of the patients. Renal insufficiency was defined as having creatinine clearance values < 40 ml/min, liver dysfunction as having serum liver enzyme levels higher than three times the upper limit of normal. The haematologic parameters were within the range that is to be expected in this stage of the disease. The CD4+ T cell counts of the patients ranged from 293 to 98/μl.

Amphothericin B was intravenously administered for two weeks (daily dose: 0.4 mg/kg body weight). Patients were required to fast overnight and up to 2 hours after having received their dose of antipyrine 1200 mg. Blood was collected by venipuncture. For assessment of the antipyrine concentrations, samples were collected prior to the administration of the drug and after 12, 24 and 36 hours. Urine samples were recovered at the following intervals: 0-12 h, 12 - 24 h, 24-48 h, and 48-72 h. All blood samples were centrifuged at 3000 g and frozen at -80 C° until analysis. The antipyrine concentrations were determined by means of high-performance liquid chromatography (HPLC), as proposed by Eichelbaum and Spannbrucker [11]. A specific radioimmunoassay (RIA) previously described by Park et al. [12] was used to determine the hydroxycortisol levels. The 17-ketocorticosteroid concentrations were measured colorimetrically. The elimination constant was calculated from the drug levels by means of a linear least squares regression line. The total body clearance (cL_t)/F was calculated by dividing dose by $AUC_{0-\infty}$. (F represents the fraction of drug absorbed). The volume of distribution (Vd)/F was calculated from the elimination constant. The peak plasma concentration (Cmax) was directly determined from the data, and tmax, e.g., the time to reach the peak plasma concentration, was derived from the respective data point.

All results were expressed as *mean* values and *standard deviation* (SD). Normally distributed data were analyzed for significant differences using the 2-tailed Student`s t-test for paired samples. p values of less than 0.05 were considered significant., p values of less than 0.01 very significant (including calculation of the Bonferroni formula).

Results

Therapy with Am-B was well tolerated and all included patients finished the study. No clinically adverse effects were observed. The following significant changes were found after Am-B treatment ($P < 0.01$): increase of antipyrine half-life and the area under the plasma concentration-time curve; decrease of the total body clearance and decrease of the renal clearance of antipyrine metabolites (norantipyrine, 4-hydroxyantipyrine, hydroxymethylantipyrine); decrease of urinary 6-β-hydroxycortisol excretion and the ratio of 6-β-hydroycortisol to 17-hydroxycorticosteroids. Statistically not significant was the volume of distribution of antipyrine after Am-B as well as the decrease of 17-hydroxycorticosteroids after Am-B (n.s.). For all parameters that were normally dis-

Table 1. a) The impact of amphothericin B (Am-B) treatment on the half-life (T1/2), the volume of distribution (Vd), the total body clearance (Cl_t), and the area under the plasma concentration-time curve (AUC) of antipyrine. b) The impact of AM-B on the renal clearance of antipyrine and its metabolites [antipyrine (Anti Cl_r), norantipyrine (NorA Cl_m), 4-hydroxyantipyrine (OHA Cl_m), 3-hydroxymethylantipyrine (HMA Cl_m)].

a)	**$T_{1/2}$** (h)	**Vd** (l)	**Cl_t** (ml/ min)	**AUC** (mg min/ml)
At baseline	12.4 ± 3.01	55.6 ± 11.9	61.2 ± 11.9	27.9 ± 6.73
After Am-B therapy	16.8 ± 5.45 $P < 0.01$	51.3 ± 8.72 n.s	43.7 ± 9.42 $P < 0.01$	38.1 ± 9.46 $P < 0.01$
b)	**Anti Cl_r** (ml/ min)	**NorA Cl_m** (ml/min)	**OHA Cl_m** (ml/min)	**HMA Cl_m** (ml/ min)
At baseline	1.94 ± 0.49	7.45 ± 2.01	15.4 ± 4.98	4.31 ± 0.97
After Am-B therapy	1.82 ± 0.58 n.s.	5.31 ± 1.65 $P < 0.01$	10.3 ± 3.41 $P < 0.01$	3.65 ± 0.91 $P < 0.01$

Data are expressed in means ±SD; n.s. = non-significant

Table 2. The impact of amphothericin B (Am-B) treatment on the urinary excretion of 6-β-hydroxycortisol (6-β-OHC) and 17-hydroxycorticosteroids (17-OHC) [6-β-hydroxycortisol to 17-hydroxycorticosteroids ratio (6-β/17-OHC)]

	6-β-OHC (μg/24h)	17-OHC (μg/24h)	6-β/17-OHC (%)
At baseline	453 ± 87.4	5169 ± 1917	8.8 ± 2.92
After Am-B therapy	298 ± 81.5P $P < 0.01$	5169 ± 1884 n.s.	6.4 ± 1.58 $P < 0.01$

Data are expressed in means ± SD; n.s. = non-significant

tributed we used the 2-tailed Student`s t-test for paired samples for all calculations. *Mean values* and *standard deviation* (SD) are detailed in Tables 1 and 2.

Discussion

Frequently, antifungal agents and antiretroviral drugs are used concomitantly in the treatment of HIV-infected patients. In many in vivo studies it has been shown that there are various exogenous factors (e.g., smoking, caffeine) and a great number of drugs, which influence the enzyme activity of the P450 monooxygenase system of the liver [12, 13]. In several in vitro studies, inhibitory effects on certain hepatic cytochromes P450 enzymes have been observed after application of antifungal drugs such as fluconazole and ketoconazole [14-17].

Inselmann and co-workers [6-8] intensively studied the influence of conventional Am-B on hepatic microsomal enzyme function in rats ex vivo. In their first studies, they demonstrated that AM-B (3 mg/Kg body weight, intravenously) given daily for three days [6] or for four days [7] significantly decreases hepatic cytochrome P450 content and function in the rat. By contrast, their results indicate that Am-B has no effect on glucose-6-phosphatase in vivo [6]. These results have been confirmed by a recent study of Inselmann and colleagues [8] in which the effects of liposomal Am-B and conventional Am-B on the hepatic microsomal enzyme function have been investigated in the rat ex vivo. Conventional Am-B caused a significant decrease in the concentration of hepatic microsomal cytochrome P450, however, liposomal Am-B had no adverse effects on microsomal hepatic enzymes and the antipyrine clearance [18].

Reduced amount of hepatic cytochrome P450 may be caused by either reduced synthesis or increased catabolism. The latter could be anticipated if Am-B has a direct toxic effect on hepatocytes. However, we consider this possibility unlikely, since severe hepatotoxicity is a rare side effect of AM-B therapy in humans and even chronic AM-B treatment in rats for three months has no effect on the serum transaminases or the alkaline phosphatase [19, 21]. Although an enhanced degradation of cytochrome P450 cannot absolutely be ruled out, it is more likely that low cytochrome P450 concentrations following AM-B treatment are due to a selective inhibition of cytochrome P450, possibly resulting from xenobiotic interaction. Thus Am-B or its vehicle sodium deoxycholate, impairs certain monooxygenases located on the endoplasmic reticulum, probably resulting in a decrease of hepatic protein synthesis [7].

In order to evaluate whether Am-B treatment impairs metabolic liver function in HIV-infected patients, we employed well-established techniques (antipyrine clearance, 6-β-hydroxycortisol) that are considered to be a differential measure of the enzyme activity of the cytochrome P450 system. [9, 21, 22]. A decreased antipyrine clearance indicates a reduced overall Cytochrome P450 activity. Endogenous cortisol metabolism serves as a biomarker for CYP3A4 activity.

In comparison to the dosage regimen reported by Inselmann and co-coworkers [6-8], we used relatively low Am-B doses. Nevertheless, the alterations of the antipyrine clearance and 6-β-hydroxycortisol output observed our trial strongly indicate that treatment with Am-B has a significant depressive effect on the hepatic cytochrome P450 system in HIV patients. The effect of Am-B on CYP3A4 seems to be highly relevant in patients treated with antiretroviral drugs, as most protease inhibitors are metabolized via this pathway. As observed with other drugs, which are metabolized by CYP3A4, the concomitant application of amphotericin B may lead to interactions with antiretroviral protease inhibitors. This should particularly be taken into account for the dosage regimen during the therapy of HIV-infected patients also receiving amphotericin B.

A careful drug monitoring system seems advisable, especially for HIV-patients receiving proteinase inhibitors. Instead of conventional Am-B, the use of liposomal Am-B therapy should be preferred in HIV patients with HAART in order to avoid an additive impairment of the metabolic liver function.

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