CLINICAL MYCOLOGY

The effect of nebulised pentamidine on the concentration of intra-oral *Candida albicans* in HIV-infected patients

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Summary. This prospective study investigated whether oral candidal carriage in HIV-infected patients was altered by pentamidine therapy. Repeated oral rinses were taken from 56 HIV-positive patients over a 2-year period. Oral candidal carriage was investigated in two groups of patients, one receiving prophylactic pentamidine therapy and the other not receiving regular prophylaxis. Patients receiving pentamidine had lower concentrations of *Candida albicans* intra-orally than patients who did not receive it. Furthermore, patients who received pentamidine at one stage of the study, but not another, also had lower concentrations of *C. albicans* intra-orally when receiving pentamidine. These findings indicate that pentamidine is useful as a local agent for prophylaxis against intra-oral candidosis in HIV-infected patients.

Introduction

Oral candidosis is a common feature of symptomatic infection with the Human Immunodeficiency Virus (HIV)¹ and of AIDS.² The adhesion of *Candida* spp. to the oral mucosa is a necessary pre-requisite for successful colonisation and infection and is increased in certain disease states.³ In HIV-positive individuals, as in some cancer patients, oral candidosis may predispose to fungaemia⁴ or infection of the oesophagus⁵ or epiglottis,⁶ although this link is tenuous. Nevertheless, antifungal therapy is frequently prescribed for HIV patients with oral candidosis.⁶

Traditionally, the treatment of oral candidosis was with polyene or imidazole antifungal agents but this has changed recently with the advent of the triazole drugs.⁷ It appears that some triazole drugs have anticandidal adhesion activity at the oral mucosa as well as direct antifungal activity.⁸ Non-specific antifungal treatments have also been employed, including gentian violet⁹ and chlorhexidine gluconate.¹⁰

HIV-infected individuals are at risk from *Pneumocystis carinii* pneumonia and often receive nebulised pentamidine isoethionate¹¹ which was developed initially as a trypanocidal agent.¹² The protozoan versus fungus debate on the taxonomy of *Pneumocystis carinii* has existed for many years although recent reports tend to favour the latter. The evidence for this view includes detailed ultrastructural studies,¹³ rRNA sequences shared with *Saccharomyces cerevisiae*,^{14, 15} structural characteristics of dihydrofolate reductase

and thymidylate synthetase^{16, 17} and the possession of epitopes identified by a monoclonal antibody that are also present in *S. cerevisiae*, *C. lipolytica* and *C. krusei*.¹⁸ However, the exact taxonomic relationships of *P. carinii* remain unresolved, although this may improve once the rRNA sequences of more eukaryotic micro-organisms are known. Although there were early reports of the antifungal activity of pentamidine,^{19, 20} and recent reports of growth inhibition of *C. albicans* *in vitro*,²¹ there seem to be no data on the antifungal effect of pentamidine *in vivo*. This prospective study was performed to assess the effect of nebulised pentamidine on oral candidal carriage in HIV-infected patients.

Materials and methods

Patient selection

The patients in the study comprised 56 HIV-positive individuals attending an Infectious Diseases Out-Patients Department. There were 52 males and four females. The age range was 21–58 years, with the majority aged 21–40 years. The patients had acquired their disease as follows: 40 by homosexual contact; six through intravenous drug use; nine as a result of receiving a transfusion of infected blood; and one from bisexual contact. According to the CDC classification, 24 patients were CDC II, eight were CDC III and a further 24 patients were CDC class IV. Informed consent was received from all patients at the first visit. A full history and intra-oral and extra-oral examination was performed. The patients were reviewed at

Table. Intra-oral concentration of *C. albicans* in saliva of HIV-infected patients

Treatment	Number of visits	Median concentration	
		cfu/ml	log ₁₀ cfu/ml
Off pentamidine	49	2600	7.86
On pentamidine	128	1800	7.50

6-weekly to 3-monthly intervals for a period of 2 years. On each subsequent visit, the history was updated and the examination was repeated. An oral rinse, as described below, was performed on each visit. This was done before the inhalation of pentamidine in those patients receiving pentamidine prophylaxis.

Estimation of intra-oral *C. albicans* concentration

The concentration of intra-oral *C. albicans* was estimated by the oral rinse culture technique.²² A 10-ml sample of phosphate-buffered saline was swirled in the mouth for 1 min and then expelled into a sterile glass container. Within 1 h, the samples were plated with a spiral plater and inoculated on to Sabouraud's agar. The plates were incubated at 37°C for 48 h aerobically. The number of colonies present on the agar were counted to estimate the concentration of *C. albicans* present intra-orally.

Statistical methods

The data analysed were the concentrations of *C. albicans* at each visit as well as the time from the patient's first HIV-positive test to that visit. Also recorded and analysed were whether or not the patient had been on pentamidine for a minimum of 4 weeks before the visit and the patient's weight, T4:T8 ratio and CDC classification at first visit.

A generalised linear model with the logarithm of the *C. albicans* concentration as the response variable was fitted with all the other variables above as possible explanatory variables in various choices of sub-model and, in particular, allowing for a subject effect. Appropriate F tests were used to assess the significance of each of these possible explanatory variables on the log concentration with all other variables already fitted in the model.

Results

The results of the effect of pentamidine on the concentration of intra-oral *C. albicans* are summarised in the table and indicate that pentamidine reduces the concentration of *C. albicans* ($p < 0.001$). Furthermore, of 11 patients on pentamidine at some point in the study and off prophylaxis on at least one other occasion, nine of the 11 showed higher values when pentamidine was absent and this was confirmed by statistical analysis, which showed a significant pentamidine effect ($p < 0.005$). There was no correlation between intra-oral candidal levels and either the patients' weight or T4:T8 ratio.

Discussion

Pentamidine is an aromatic diamidine that was developed primarily as a trypanocidal drug¹² and, later, was found to be useful in bacterial diseases.¹⁹ Nebulised pentamidine isoethionate has been shown to prevent *Pneumocystis carinii* pneumonia (PCP) or to delay relapse in HIV-infected individuals.¹¹ However, the mechanism of action of pentamidine is not completely understood. The antifungal activity of the diamidines was first reported by Elson in 1945 when he presented the result of in-vitro tests with propamidine hydrochloride on several fungi, known to be pathogenic to man.¹⁹ Christison *et al.* showed that the aromatic diamidines are also active in the treatment of blastomycosis, actinomycosis, histoplasmosis, cryptococcosis and chromoblastomycosis.²⁰ St Germain, in 1990,²¹ reported that pentamidine significantly inhibited the growth of strains of *C. albicans* from HIV-positive patients *in vitro*. The efficacy of nebulised pentamidine for the treatment of oral candidosis in HIV-infected patients *in vivo* is supported by our present findings.

Several studies have reported the problems of failed response to antifungal therapy in HIV-positive individuals with oral candidosis.²³ Systemic preparations are used frequently and occasionally these are administered parenterally in an attempt to control the infection. As nebulised pentamidine is established as effective prophylaxis against PCP and is relatively easy to administer, it reduces the intake of systemic preparations in patients who are frequently receiving multiple drug therapies, and from our observations, significantly reduces the intra-oral concentration of *C. albicans* in HIV-positive patients.

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