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Ketoconazole in atopic dermatitis: therapeutic response is correlated with decrease in serum IgE

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Abstract The prevalence of specific IgE antibodies to the yeasts *Pityrosporum orbiculare* and *Candida albicans* was investigated in adult patients with atopic dermatitis (AD) or with seborrhoeic dermatitis and in healthy controls by means of the radioallergosorbent test (RAST). Of 63 AD patients, 28 (44%) had IgE antibodies to *P. orbiculare* and 21 (33%) to *C. albicans*. This is highly significant, since no antibodies were found in sera from other patients or controls. With the intention to treat, 20 patients with AD and a positive RAST to *P. orbiculare* were given ketoconazole 200 mg daily for 2 months and 200 mg twice weekly for further 3 months. The clinical scores improved during treatment with a reduction in the levels of specific IgE to *P. orbiculare* and total serum IgE. However, there were no correlations between clinical score and serum levels of *P. orbiculare*-specific IgE. *C. albicans*-specific IgE, on the other hand, correlated both with clinical score and with total serum IgE.

Introduction

The lipophilic yeast *Pityrosporum orbiculare* (*ovale*) is involved in the pathogenesis of pityriasis versicolor [1], *Pityrosporum* folliculitis [2, 3] and seborrhoeic dermatitis [4]. Recent reports also indicate that immune responses to *Pityrosporum* antigens may occur in patients with atopic dermatitis (AD). Thus it has been demonstrated that a substantial proportion of patients with AD have a positive skin prick test [5–7] and after scarification also a positive patch test [8] to *Pityrosporum* allergens. In a double-blind

trial of AD patients with the head and neck-type distribution and a positive skin prick test to *P. orbiculare*, oral ketoconazole therapy has been shown to significantly reduce the intensity of the dermatitis, but without changing in the prick test reaction or total serum IgE concentration [9].

The aims of the present study were to identify adult patients with serum IgE specific for *P. orbiculare*, and to determine whether extended oral ketoconazole therapy could influence the dermatitis and the level of specific IgE antibodies to *P. orbiculare* as well as total serum IgE levels, and other parameters of immune cell activity.

Materials and methods

Adult patients with atopic and seborrhoeic dermatitis attending the outpatient clinic of the Department of Dermatology and Venereology at the University Hospital, Umeå, were investigated. Patients from the venereal disease clinic served as healthy controls. The study was approved by the local Ethics Committee and was performed during winter 1990/91 and 1991/92.

Experimental design

During the first winter season serum samples were obtained from patients with AD, seborrhoeic dermatitis and from controls. The presence of IgE antibodies to *P. orbiculare* and *C. albicans* antigens was measured using the radioallergosorbent test (RAST) as previously described [10], using commercially available Phadebas RAST kits (Pharmacia Diagnostics, Uppsala, Sweden) and following the recommendations of the manufacturer. The sera were tested in duplicate with *P. orbiculare* (m70) discs or *C. albicans* (m5) discs, respectively, and the results expressed in Phadebas RAST units (PRU/ml).

The following winter, 20 AD patients with significant levels of IgE antibodies to *P. orbiculare* were selected to enter an open ketoconazole treatment study. At baseline the dermatitis activity was scored according to method used by Sowden et al. [11]. In brief, six clinical features (erythema, purulence, excoriation or crusting, dryness or scaling, cracking or fissuring, and lichenification) were graded at six defined body sites on a scale of 0 (none) to 2 (severe). This gave a total body (global) disease activity score (maximum 72) and a head and neck area score (maximum 12). Serum samples were obtained for analyses of serum IgE (IgE RIA; Pharmacia Diagnostics; normal value < 149 kU/l), IgE antibodies to *P. orbiculare* and *C. albicans* (RAST, Pharmacia Diagnostics), soluble in-

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terleukin-2 receptor (sIL-2R/CD25; T Cell Diagnostics, Cambridge, Mass., USA) and eosinophil cationic protein (ECP, RIA, Pharmacia, Uppsala, Sweden). The patients were then treated with ketoconazole (Fungoral, Janssen Pharma, V Frölunda, Sweden) 200 mg daily for 2 months followed by an assessment of skin disease activity and repeated blood samples. After this treatment period the ketoconazole dose was reduced to 200 mg twice a week on consecutive days for 3 months followed by another assessment of skin disease activity and blood samples for evaluation of immune cell activity. During the treatment period the liver function was followed by repeated determinations of liver enzymes.

Patients with AD

This group comprised 63 patients (22 males and 41 females) with AD diagnosed according to the criteria of Hanifin and Rajka [12]. Allergic rhinoconjunctivitis was present in 22 patients and asthma bronchiale in 15, while another 10 patients had a history of allergic rhinoconjunctivitis and 4 patients a history of asthma. The remaining 20 patients suffered from AD alone without allergic respiratory disease (ARD). The median age of the patients was 26 years with a range of 16 to 51 years.

Patients with seborrhoeic dermatitis

This group comprised 11 patients (8 males and 3 females) with skin lesions on the trunk as well as on the scalp and facial area. They were free from ARD and their median age was 30 years with a range of 22 to 45 years.

Healthy controls

The control group comprised 39 patients (22 males and 17 females) without specific skin disease attending the venereal disease clinic for a check-up and enrolled in the HIV testing programme and without disease. Their median age was 23 years with a range of 17 to 35 years.

Ketoconazole treatment group

This groups comprised 20 patients (10 males and 10 females) with AD and IgE antibodies to *P. orbiculare* (5 patients with RAST class 4, 6 patients with class 3 and 9 patients with class 2). Specific serum IgE antibodies to *C. albicans* were present in 14 patients (1 patient with RAST class 4, 8 patients with class 3, 4 patients with class 2, and 1 patient with class 1). All patients except one had an elevated total serum IgE (> 149 kU/l) and 12 patients had a serum IgE exceeding 1000 kU/l. The median age was 35 years with a range of 20 to 52 years. Four patients suffered from isolated AD, while ten had additional ARD, allergic rhinoconjunctivitis and/or asthma bronchiale, and six had a history of past ARD. The disease activity varied between individual patients from mild to severe dermatitis. Most patients, however, had a moderate dermatitis as indicated by the mean global score.

Statistics

Wilcoxon's signed rank's test for paired samples was used for assessment of differences between treatment groups, while Spearman's rank correlation test and Fisher's exact test were applied for the evaluation of the interrelationship between the observed parameters.

Results

Screening of IgE antibodies to *P. orbiculare* and *C. albicans*

Serum samples from 74 patients and 39 healthy controls were analysed by RAST. In the AD population, 28 of 63 patients (44%) had specific IgE antibodies to *P. orbiculare* while none of 11 patients with seborrhoeic dermatitis and none of 39 controls had positive RAST tests. Specific IgE antibodies to *C. albicans* were found in 21 of 63 AD patients (33%), but not in patients with seborrhoeic dermatitis or in healthy controls. The correlation between IgE antibodies to *P. orbiculare* and *C. albicans* was strong: 20 of 28 patients with IgE antibodies to *P. orbiculare* had antibodies to *C. albicans* and 20 of 21 patients with IgE antibodies to *C. albicans* also had positive RAST tests to *P. orbiculare* (Fisher's exact test; $\chi^2 = 29.9$; $P < 0.001$). The presence of IgE antibodies to *P. orbiculare* was correlated with elevated serum IgE since 27 of 28 patients with IgE antibodies to *P. orbiculare* had elevated total serum IgE and 27 of 40 patients with elevated serum IgE also had specific IgE antibodies to *P. orbiculare* ($\chi^2 = 21.1$; $P < 0.001$). The serum IgE levels of the 13 patients who were RAST-negative to *P. orbiculare* varied between 151 and 18 900 kU/l. Table 1 shows the distribution of elevated serum IgE levels and the presence of specific IgE antibodies to *P. orbiculare* and *C. albicans* in subgroups of AD patients with and without ARD, respectively. In this limited population of AD patients, however, there were no significant differences between the two subgroups.

Effect of ketoconazole therapy

Of the 20 patients, 18 completed the ketoconazole treatment schedule for 5 months. Two patients withdrew for personal reasons. No adverse effects of treatment were observed and liver function tests were normal throughout. The patients were allowed to continue their topical treatment during the study period.

Most patients reported a good to moderate response of the dermatitis to ketoconazole 200 mg daily during the 2-month period with no further improvement when the dose was reduced to 200 mg twice weekly. This subjective evaluation corresponded well with a significant decrease

Table 1 Increased serum IgE levels and presence of specific IgE antibodies to *P. orbiculare* and *C. albicans* in 63 AD patients with and without allergic respiratory disease (ARD)

	With ARD (n = 43)	Without ARD (n = 20)
Serum IgE > 149 kU/l	31 (72%)	9 (45%)
<i>P. orbiculare</i> IgE	20 (47%)	8 (40%)
<i>C. albicans</i> IgE	17 (40%)	4 (20%)

Table 2 Clinical scores and laboratory data of 18 AD patients at baseline and after ketoconazole treatment for 2 and 5 months, respectively (mean $\pm$ SE)

	Baseline	2 months	5 months
Global score	17.0 $\pm$ 2.2	11.8 $\pm$ 1.7**	10.4 $\pm$ 1.4**
Headneck score	4.6 $\pm$ 0.6	2.9 $\pm$ 0.5**	2.8 $\pm$ 0.4**
IgE (kU/l)	3334 $\pm$ 802	3173 $\pm$ 936	2518 $\pm$ 657*
<i>P. orbiculare</i> IgE (PRU/ml)	19.6 $\pm$ 4.3	12.9 $\pm$ 3.4*	13.2 $\pm$ 4.0*
<i>C. albicans</i> IgE (PRU/ml)	3.3 $\pm$ 1.5	3.1 $\pm$ 1.6	2.2 $\pm$ 1.0
sIL-2R/CD25 (U/ml)	555 $\pm$ 34	543 $\pm$ 36	542 $\pm$ 47
ECP (μ g/l)	18.7 $\pm$ 3.7	20.7 $\pm$ 2.8	13.5 $\pm$ 2.0

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus baseline; Wilcoxon's rank test for paired samples

Table 3 Correlation between serum levels of specific IgE antibodies to *P. orbiculare* and *C. albicans* and other measured parameters in 18 ketoconazole-treated AD patients (values shown are Spearman's rank correlation coefficient, r)

	Baseline	2 months	5 months
<i>P. orbiculare</i> IgE vs:			
Global score	-0.03	0.05	0.17
Headneck score	-0.02	-0.12	0.44
Total IgE	0.54*	0.36	0.56*
sIL-2R/CD25	-0.24	-0.03	-0.15
ECP	0.01	-0.16	0.56*
<i>C. albicans</i>	0.61*	0.23	0.48*
<i>C. albicans</i> IgE vs:			
Global score	0.56*	0.79**	0.55*
Headneck score	0.22	0.44	0.46
Total IgE	0.84***	0.83***	0.79**
sIL-2R/CD25	0.37	0.31	0.13
ECP	0.14	0.27	0.23

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table 4 Correlation between clinical scores and immune cell activities in 18 ketoconazole-treated AD patients (values shown are Spearman's rank correlation coefficient, r)

	Baseline	2 months	5 months
Global score vs:			
Headneck score	0.52*	0.66**	0.47
Total IgE	0.49*	0.68**	0.38
<i>P. orbiculare</i> IgE	-0.03	0.05	0.17
<i>C. albicans</i> IgE	0.56*	0.79**	0.55*
sIL-2R/CD25	0.53*	0.44	0.49*
ECP	0.14	0.07	0.20
Headneck score vs:			
Total IgE	0.19	0.49*	0.56*
<i>P. orbiculare</i> IgE	-0.02	-0.13	0.44
<i>C. albicans</i> IgE	0.22	0.44	0.46
sIL-2R/CD25	0.51*	0.81***	0.42
ECP	0.06	0.22	0.36

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

in both the global disease activity score and the head and neck score (Table 2). Some of the patients were able to stop treatment with topical steroids during the ketoconazole period. Several patients wanted to continue with ke-

toconazole on a daily basis when the study was completed.

Table 2 also shows that ketoconazole therapy decreased both the level of serum IgE and the serum concentration of specific antibodies to *P. orbiculare*. The concentration of serum IgE antibodies to *C. albicans* was also diminished, but not significantly. The serum levels of soluble IL-2R/CD25 was not significantly influenced in this study. The serum concentrations of ECP showed unchanged levels after 2 months of ketoconazole but a significant drop subsequently when the medication was continued intermittently for another 3 months.

Statistical treatment of the data at baseline showed a correlation between the global clinical score and serum IgE, specific IgE antibodies to *C. albicans* and serum levels of sIL-2R/CD25, respectively. Surprisingly the head and neck score was not correlated with *P. orbiculare*-specific IgE. Table 3 shows the correlation between specific yeast IgE antibodies and other disease parameters and Table 4 shows the correlation between clinical score and immune cell activities.

Discussion

The role of skin surface microorganisms, both bacteria and yeasts, in atopic dermatitis has recently been reappraised [13]. Our results show that IgE antibodies to *P. orbiculare* were frequent in adult patients with AD and were present in 44% of the patients. In this fraction of AD patients the prerequisite for IgE-mediated immediate and late phase skin reactions was therefore present. This finding seems to be rather specific for AD, since healthy controls, patients with seborrhoeic dermatitis and patients with ARD but without AD lacked these antibodies [6, 7, 10, 14, 15]. Whether the IgE antibodies are the primary cause of the dermatitis or a secondary event to the dermatitis following antigen stimulation through a disrupted skin barrier is still unsettled.

One of the aims of this study was to see whether ketoconazole could improve the dermatitis, especially the head and neck score, and whether this improvement correlated with a decrease in specific antibodies to *P. orbiculare*. As shown in Table 3 there was no such correlation. Instead consistent correlations existed only between *P. orbiculare* IgE and total IgE and *C. albicans* IgE, respectively. The correlation between *P. orbiculare* IgE and total

serum IgE was, however, present at baseline and after 5 months, but not after 2 months, of daily therapy with oral ketoconazole.

C. albicans-specific IgE behaved differently and showed a strong correlation not only with total IgE but also with the global clinical score. In addition the clinical scores (Table 4) correlated with each other, which is reasonable, and with total IgE, which was reported long ago [16]. The correlation between disease activity in AD and serum levels of sIL-2R/CD25 has recently been reported [17], and our findings were similar. In this study, however, we were not able to confirm that the serum concentration of ECP correlated with the severity of AD as described elsewhere [18].

An interesting issue is the importance of *C. albicans*-specific IgE in AD. Whether the immunization occurs through the epidermis or via the epithelia of the gastrointestinal or genital tract or the respiratory organs is not known. It cannot be excluded that internal colonization with *C. albicans* and continuous release of antigens may, in sensitive patients, be responsible for the chronic nature of the dermatitis [19]. Since there is a considerable serological cross-reactivity between *C. albicans* and other yeasts, the 'specific' antibodies may represent multiple yeast sensitivities [20–22].

Ketoconazole is a drug that, besides its established antifungal potency, may also have an antiinflammatory effect through an inhibitory action on 5-lipoxygenase [23]. Imidazole antimycotics including ketoconazole also inhibit T-cell proliferation in vitro [24, 25]. Therefore the effects of ketoconazole on clinical scores and immune cell parameters in this study are far from explained. Furthermore, we have not investigated the ability of ketoconazole, with the dose regimen used in this study, to reduce or eliminate the antigenic load derived from the yeasts *P. orbiculare* and *C. albicans* [26]. With the results presented here at hand, further studies are highly desirable involving AD patients with and without IgE antibodies to *P. orbiculare* and *C. albicans* and using placebo-controlled antifungal treatment protocols.

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