exacerbation was the outcome in 2 cases. The number of treatment sessions, the maximum individual dose, and the total UVA dose varied considerably from case to case (Table I). Fewer than 20 treatments were accomplished in patients 1 and 8, who interrupted the treatment because of an exacerbation. Other side effects were smarting and erythema in patient 1 at a dose of 1.2 J/cm² and in patient 10 at 12 J/cm²; patient 7 experienced a severe burning with large bulla formation at 4 J/cm².

DISCUSSION

This study records poor results with topical psoralen application in the PUVA treatment of palmoplantar pustulosis, even with up to 128 treatments and total doses of up to 144–348 J/cm². These results contrast both with the favourable effect documented with topical psoralens and UVA in the treatment of psoriasis vulgaris (1, 6), and the therapeutic success reported with oral methoxalen and UVA in the treatment of PPP (2, 3, 4, 5).

In the case of 8-MOP bath application the inadequate effect might, at least partially, have resulted from a depletion of the drug from the skin during the prolonged irradiation times (up to 40 min) used in the higher UVA dosage range. After a trioxsalen bath, the photosensitivity of body skin has been shown to diminish to 50% of the starting value during the first 30 min after bathing and to 25% during the next 30 min (1). In the case of a psoralen ointment, therapeutic drug concentrations may be retained in the skin for somewhat longer periods. In one study, after removal by blotting paper of an 0.01% trioxsalen ointment, one-fourth of the photosensitivity was retained at 2 h (7). That effective sensitization of the skin was actually obtained in our study, can be inferred from the fact that 3 of the patients developed painful erythema of the treated skin areas, one developing a bullous phototoxic reaction; in only one of these patients was a favourable clinical response obtained, however.

Recently, Murray et al. reported a study in which 7 out of 15 PPP patients cleared on a treatment consisting of the application of an 0.15% 8-MOP oil emulsion and 30 irradiations with a mean clearing dose of 8 J/cm² (5). In our series 5 patients were treated with 8-MOP ointment and UVA, 4 receiving more than 30 treatments; the maximal UVA dose varied from 1.2 to 12 J/cm². None of the 5 patients showed any improvement. The discrepancy of these results and those of Murray et al. (5), could be due to chance alone, to different vehicles, different application times (60 min vs. 90 min), different UVA dosage schemes, different frequently of treatment (3 vs. 4 times a week), or any combination of these factors. Clearly, more studies are required to delineate the efficacy of topical 8-MOP and UVA treatment of palmoplantar pustulosis. In particular, systemic versus topical psoralen, psoralen solution vs. psoralen ointments, and different application and irradiation schedules should be evaluated.

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Solubility of Antimycotics: A Problem in *in vitro* Experiments

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Abstract. An investigation of how miconazole nitrate dissolved in dimethylformamide in initial concentrations of 1 000, 500, and 100 μg/ml may alter its concentration with

Table I. *Concentration of miconazole nitrate, in three different stock solutions, in relation to time*

Time (hours)	Miconazole nitrate (% of initial-concentration) in stock solutions (μg/ml)		
	1 000	500	100
0	100.0	99.0	98.2
1	101.2	99.0	102.1
2	97.8	100.0	99.5
3	100.0	96.7	98.3
4	101.4	95.8	104.3
8	82.8	98.6	96.6
24	73.0	77.3	97.1

storage time, is presented. The determination of miconazole nitrate was performed with high pressure liquid chromatography. After 8 hours the 1 000 μg/ml solution was milky and the concentration of miconazole had fallen significantly. It was concluded that test agar plates or agar tubes must be made with freshly prepared and clear stock solutions, otherwise false MIC's may be obtained.

Key words: Antimycotics; miconazole; solubility

Several antimycotics are not water-soluble and this raises several problems when *in vitro* tests are performed. In an earlier investigation of the *in vitro* antimycotic activity of imidazoles we mentioned this problem (1). If an antimycotic is only slightly soluble in water, it is necessary to dissolve it first in an organic solvent for *in vitro* studies. This raises two problem. One is the risk of precipitation of the antimycotic when water is added; the other is the possible antimycotic effect of the organic solvent itself.

To solve the first problem a determination of the concentration of miconazole nitrate in different stock solutions in relation to time was performed.

MATERIAL AND METHODS

Preparation of stock solutions

Miconazole nitrate was obtained from Janssen Pharmaceutica, Beerse, Belgium. It was dissolved in dimethylformamide (DMF) and then diluted with distilled water to give three stock solutions with a concentration of DMF of 20% and miconazole nitrate concentrations of 1 000, 500, and 100 μg/ml.

Determination of miconazole nitrate concentration

The concentration of miconazole nitrate in the stock solutions was determined by high pressure liquid chromatography (HPLC) at 220 nm. Samples from the three stock solutions were analysed as soon as they were made up (0) and after 1, 2, 3, 4, 8, and 24 hours.

A reversed phase RP-18 column 30 cm long with an internal diameter of 4.6 mm was used. The elution was carried out isocratically with a mixture of 65% of a 0.5% aqueous ammonium acetate solution and 35% of a solution containing acetanilide and 0.2% *N*-(1-methylethyl)-2-propamine. Peak areas were measured by computer integration. Retention time of miconazole nitrate was approximately 9 minutes. Miconazole nitrate concentrations were determined using the external standard calibration procedure. Therefore reference solutions of miconazole nitrate in methanol were made corresponding to the concentrations of the stock solutions. The stock solutions were coanalysed with corresponding reference solutions at the intervals mentioned earlier. By injecting aliquots of 50 μl. variations of about 2% were obtained for the 1 000 and 500 μg/ml solutions and nearly 5% for the 100 μg/ml solution. Therefore the samples were analysed twice and the mean value taken. To minimize differences in peak area and also to avoid differences in computing large or small areas, the peaks of the 100, 500, and 1 000 μg/ml solutions were attenuated to 0.2, 1, and 2 respectively.

RESULTS

The concentrations of miconazole nitrate in the three stock solutions in relation to time are shown in Table I. After 8 hours there was a precipitate in the solutions with 1000 μg/ml and only 82.8% of the initially present miconazole nitrate was recovered by HPLC. After 24 hours only 73.0% of the initial concentration was found. In the solution with 500 μg/ml only 77.3% was found after 24 hours. In the solutions with a lower than expected concentration of miconazole nitrate a visible precipitate was always seen.

DISCUSSION

When an antimycotic with a low water solubility is added to a water-phase there is a risk of precipitation. In an earlier investigation we mentioned this problem (1). The present investigation shows that miconazole nitrate dissolved in DMF is not stable. Even when a precipitate of miconazole nitrate in the test bottles was hardly visible, the concentration was lower than expected. This may be due to the formation of crystals and to miconazole nitrate being precipitated on the wall of the bottle.

In the literature, where MIC-determinations for antimycotics of low water solubility are studied, it is often stated that "a milky solution was used". The obvious risk of this is that a false MIC is ob-

tained. Another problem is the antimycotic activity of the solvent (1, 2, 3). DMF has a rather low activity in comparison with ethanol, isopropyl alcohol, and acetone for example (1). Therefore, to obtain a reliable MIC of antimycotics with low water solubility several factors must be taken into account. The antimycotic should preferably be dissolved in a solvent with low antimycotic activity and then diluted with water to the highest concentration of the antimycotic where the solution still remains clear (1). The agar tubes or agar plates should be made up with freshly prepared and clear stock solutions. When a liquid medium is used there may be a risk of precipitation of the antimycotic on the inside of the tube. Even if the concentration of antimycotic is low the time of incubation is several days, and therefore a solid medium is preferable.

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Serum IgE Antibodies to Scabies Mite

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Abstract. Sixteen patients with scabies were examined. Eight (50%) of them had elevated serum total IgE levels. The highest level, 10 000 U/ml, occurred in a patient with Norwegian scabies. IgE antibodies specific to scabies mite were found in two of the five patient sera examined. Thus, it seems probable that an IgE-mediated reaction plays a role in the manifestations of scabies in man.

Key words: Scabies; IgE antibodies; RAST

There is evidence to suggest that immune mechanisms are activated in the course of human and animal scabies infection. Clinical symptoms appear about one month after primary infestation by scabies mite (*Sarcoptes scabiei*) but in reinfection pruritus and papulovesicles appear within 24 hours and the size of the mite population remains much lower (11, 13). Recent studies on scabies have shown IgE deposits in the dermal vessels of a scabious lesion, high levels of serum total IgE and frequent occurrence of IgE antibodies to house-dust mite (5, 6, 8), all suggesting that an IgE-mediated mechanism is involved.

The present study shows that IgE antibodies to scabies mite can be detected in the sera of patients with scabies by using the radioallergosorbent test (RAST).

PATIENTS AND METHODS

Patients

Sixteen patients with scabies, 13 males and 3 females, aged from 10 to 53 years were examined. At the time of examination the pruritus and rash had varied in duration from 1 to 6 months (mean 2½ months). Eleven of the patients had widespread scabies and one had the clinical picture of Norweigan scabies preceded by a 2-month treatment period with peroral (triamcinolone 4 mg/day) and topical steroids. One of the patients had previously been infected with scabies and one patient had a history of atopic dermatitis.

Scabies mite antigen

Human and pig scabies (*Sarcoptes scabiei* var. *suis*) mites are varieties of the same species (4). To obtain sufficient antigen for the present examinations the mites were collected from the ears of naturally infected pigs using the method of Sheahan (13). About 1 000 living mites including adults and nymphs were harvested with a needle after transfer to Petri dishes. Efforts were made to avoid contamination by epidermal debris. The mites were ground and suspended in 0.5 ml of 0.9% sodium chloride. The protein content of the solution was 176 μg/ml. The solution was stored at −20°C until use in the RAST.

RAST

The proteins in the scabies mite extract were coupled to Munktell filter paper discs (Grycksbo Pappersbruk, Sweden) activated with cyanogen bromide using a 70 μl extract per disc as described by Ceska & Lundkvist (3). These discs and the Phadebas RAST (Pharmacia Diagnostics, Sweden) reagents, including house-dust mite (*Dermatophagoides pteronyssinus*) discs, were used to determine IgE antibodies to scabies and house-dust mites. The specific serum IgE concentrations were given in both Phadebas RAST units per ml (PRU/ml) and in RAST scores as described by Lundkvist (10). For the present purpose arbitrarily defined results of 0.30 PRU/ml or