The Effect of UV-light on Pityrosporum Yeasts: Ultrastructural Changes and Inhibition of Growth

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The effect of UV-light on Pityrosporum yeasts (P.) was studied: P. yeasts cultured from the skin were spread on Dixon plates and irradiated with different UVB- and UVA-light dosages and read after three days, controls were not irradiated. Also P. yeasts, immediately after irradiation, were isolated from the plates and studied with an electron microscope. A significant growth inhibition or no growth at all was seen after 25, 50, 75 J/cm² UVA and 900 mJ/cm² UVB, a moderate inhibition after irradiation with 250 mJ/cm² UVB. The growth inhibition was paralleled by ultrastructural degenerative alterations: clumping of ribosomes and lysis of nuclei. The amount of "stacked material" in the vacuoles was diminished or they were completely empty, the cell wall remained unchanged. Our results imply that the positive effect of sunlight on seborrhoeic dermatitis may well be explained by the direct influence of UV-light on the P. yeasts. ***Key words: Seborrhoeic dermatitis; UV-light.***

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The aetiological significance of the Pityrosporum (P.) yeasts in the pathogenesis of seborrhoeic dermatitis has been established (1). An improvement of seborrhoeic dermatitis is seen after exposure to sunlight and, as known, seborrhoeic dermatitis responds well to PUVA treatment (2). The effect of UV-light on P. yeasts and other human skin microorganisms was studied by Faergemann et al.: a growth inhibition of P. yeasts after exposure to UV-light was found (3). It is possible that sunlight reduces the number of P. yeasts on the skin and by doing so a remission of seborrhoeic dermatitis is induced.

In the present investigation growth inhibition after UV-light exposure *in vitro* was investigated, also electron microscopic studies of UV-light irradiated P. yeasts were performed.

MATERIALS AND METHODS

Experiment I

Pityrosporum yeasts were cultured from material taken of the skin by using the 'tape-method', and grown on Dixon plates (4). The P. yeasts grown from these cultures were suspended in phosphate buffered saline (PBS), pH 7.4 to give solutions containing 10 cells/ml^4; 0.3 ml of these suspensions were spread with a glass rod on Dixon plates and irradiated. The plates were then incubated at 37°C and examined after 3

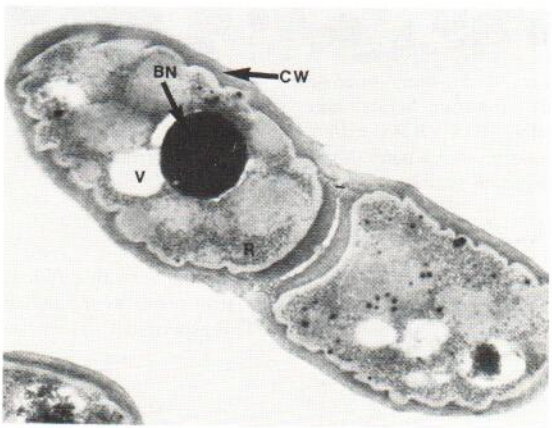

Fig. 1. Electron microscopy: (54 000×); unradiated Pityrosporum yeasts. Note ribosomes (R), undulating cell wall (CW) and vacuole (V) filled with 'stacked' material ('black nob') (BN).

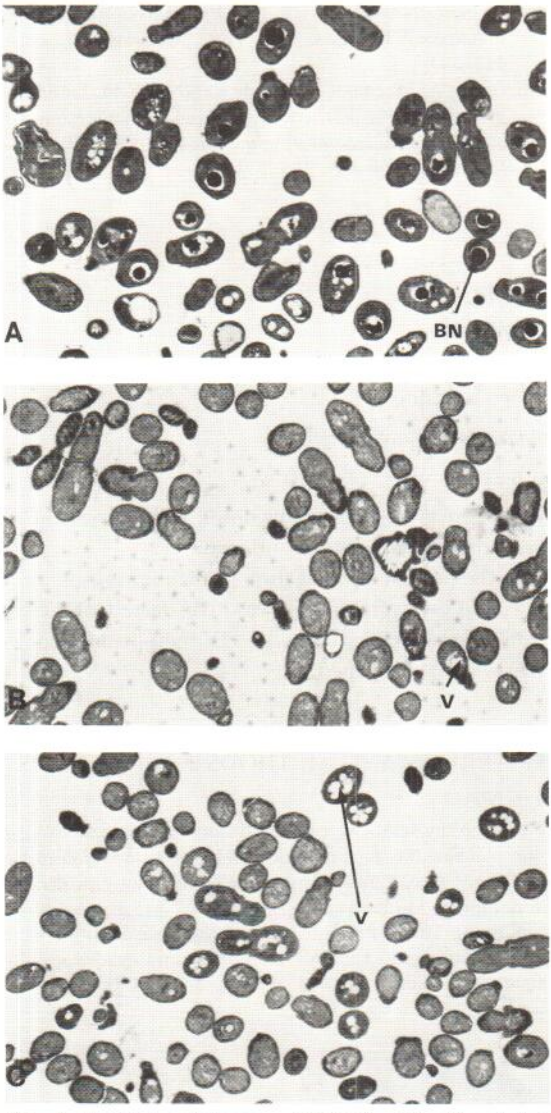

Fig. 2. Electron microscopy (4125×). (A) Unradiated Pityrosporum yeasts: note 'stacked' material in vacuoles (BN). (B) UVB 900 mJ/cm² irradiated Pityrosporum yeasts: note empty vacuoles (V). (C) UVA 75 J/cm² irradiated Pityrosporum yeasts: note empty vacuoles (V).

days. Subsequently, 7 plates were irradiated with different UVB- and UVA-light dosages. Control plates were handled the same way but not irradiated. This experiment was repeated twice.

Experiment II

P. yeasts immediately after irradiation were isolated from the plates and fixed with 5% glutaric acid aldehyde (glutaraldehyde), buffered with 0.2 M phosphate for 45 min at room temperature, then rinsed and left overnight. Post fixation in 1% osmic acid buffered with veronal acetate and with sucrose added was performed for 60 min, followed by rinsing in veronal-acetate buffer for 60 min. After dehydration the specimens were embedded in Epon 812. The ultrathin sections were cut with a LKB ultramicrotome. The preparations were examined with a Zeiss 109 electron microscope. Unradiated P. yeasts were used as controls. This experiment was done in duplicate.

Irradiation technique

The P. yeasts were irradiated with both UVB and UVA. As UVB source a Waldman UVB unit fitted with Sylvania UV6 tube lamps was used, the emission spectrum being between 290–320 nm, and the main peak at 315 nm. As UVA source a Waldman PUVA (No. 4000) fitted with Sylvania Fr90 T12 lamps, emission spectrum between 330–380 nm and the main peak at 360 nm, was used. The following dosages were used: UVB 65, 123, 250 and 900 mJ/cm²; UVA 25, 50 and 75 J/cm².

RESULTS

Experiment I

The results of UVB- and UVA-irradiation are shown in Table I: significant growth inhibition or no growth at all was seen after 25, 50 and 75 J/cm² of UVA and 900 mJ/cm² UVB irradiation; a moderate growth inhibition was seen after irradiation with 250 mJ/cm² UVB. All of the studies showed a similar pattern.

Experiment II

Electron microscopic studies of unradiated P. yeasts cultured from the skin showed characteristic features: a cell wall with undulating configuration and vacuoles filled with 'stacked material' (black nob), most prob-

Table I. *The effect of UVA- and UVB-irradiation on the growth of Pityrosporum yeasts on Dixon plates*

	Numbers of Pityrosporum colonies after irradiation Series	
UV-light dosages	I	II
UVB mJ/cm²		
65	18 240	6 840
123	17 100	5 700
250	7 980	2 280
900	50	0
UVA J/cm²		
25	855	0
50	0	0
75	0	0
Controls	15 960	8 550

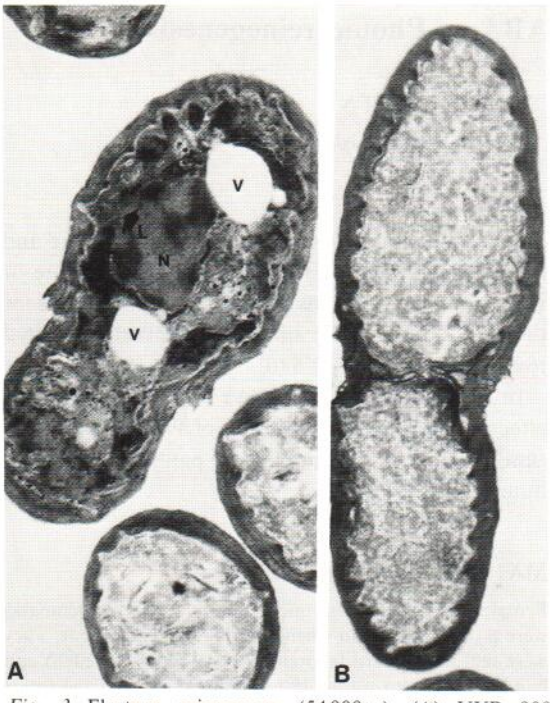

Fig. 3. Electron microscopy (54000×): (A) UVB 900 mJ/cm^2 irradiated Pityrosporum yeasts: note beginning lysis (L) of nucleus (N); clumping of ribosomes (R) and empty vacuoles (V). The cell wall stays intact. (B) UVB 75 J/cm^2 irradiated Pityrosporum yeasts: note almost complete loss of cell organelles; disintegration of nucleus can be seen, cell wall stays intact.

ably used for the cell's metabolism (Fig. 1). Furthermore, a nucleus and ribosomes were distinguished. The cells irradiated with 65, 123 and 250 mJ/cm^2 had a similar aspect. The most prominent changes were noted in the cells irradiated in the UVB-range 900 mJ/cm^2 and with 25, 50 and 75 UVA J/cm^2: clumping of ribosomes and degenerative changes of nuclei were seen. Also the amount of 'stacked material' in the vacuoles was diminished or they were completely empty, the cell wall remained unchanged (Figs. 2, 3). Identical results were obtained in the duplicate study.

DISCUSSION

Growth inhibition of P. yeasts after exposure to UV-light was found by Faergemann et al. (3) after 65, 123, 250 and 900 mJ/cm^2 UVB and 50 and 75 J/cm^2 UVA irradiation. We also found growth inhibition of P. yeasts after exposure to UV-light. However, in contrast to the results described by Faergemann, growth inhibition after 65 and 123 UVB mJ/cm^2 was not observed in our study but only growth inhibition after irradiation with 900 mJ/cm^2 and the UVA-dosages.

The growth inhibition was paralleled by ultrastructural degenerative alterations, as was shown by electron microscopic studies. These changes were seen after irradiation with 25, 50 and 75 J/cm^2 UVA and 900 mJ/cm^2 UVB. Borgers et al. studied degenerative changes in different fungi after Itroconazole treatment: with the exception of P. ovale, primary alterations were seen at the cell-periphery (5). Exposure of P. cells to different concentrations of Itraconazole resulted, when higher concentrations were used, in the disorganization of the internal organelles: absence of mitochondria and nuclei were noticed. Structural alterations at the cell periphery were not seen. We found a similar effect after exposure of P. yeasts to different UV-light dosages: degenerative alterations and disappearing of cell organelles were seen, whereas the cell membrane remained intact.

The positive effect of sunlight on seborrhoeic dermatitis is well known. The effect of UV-light on the presence of Langerhans' cells, with subsequent impairment of antigen-presenting functions, must be taken into consideration when explaining the positive effect of sunlight on seborrhoeic dermatitis. However, our results imply that UV-light has a direct influence on the P. yeasts.

From a therapeutical point of view, the killing effect of UV-light on P. yeasts is of interest, especially the strong yeast-killing properties of UVA-light.

Studies are in progress to investigate the therapeutic value of UVA irradiation in severe seborrhoeic dermatitis.

REFERENCES

1. Skinner RB, Noah PW, Taylor RM, et al. Double-blind treatment of seborrhoic dermatitis with 2% ketoconazole cream. J Am Acad Dermatol 1985; 12: 852–856.
2. Salo O von, Lassas A, et al. Behandlung der Dermatitis atopica und der Dermatitis seborrhoica mit selektiver UV-Phototherapie und PUVA. Dermatol Monatschr 1983; 169: 371–375.
3. Faergemann S, Larkö O. The effect of UV-light on human skin microorganisms. Acta Derm Venereol (Stockh) 1987; 67: 69–72.
4. Wikler JR, Haan de P, Nieboer C. The "tape-method": A new and simple method for quantitative culture of Pityrosporum yeasts. Acta Derm Venereol (Stockh) 1988; 68: 445–449.
5. Borgers M, Ven van de M. Degenerative changes in Fungi after Itraconazole treatment. Rev Infect Dis 1987; 9 (Suppl 1): 533–542.