was finalized during grant appointments to The Royal Dental College of Copenhagen and to The Danish Cancer Registry, the latter by grant no. 93/80 from the Danish Cancer Society. Computer time was made available free of charge by Northern Europe University Computing Center, Technical University of Denmark.

REFERENCES

1. Bánóczy, J.: Follow-up studies in oral leukoplakia. J Maxillofac Surg *5:* 69, 1977.
2. Einhorn, J. & Wersäll, J.: Incidence of oral carcinoma in patients with leukoplakia of the oral mucosa. Cancer *20:* 2189, 1967.
3. Gupta, P. C. Mehta, F. S., Daftary, D. K., Pindborg, J. J., Bhonsle, R. B., Jalnawalla, P. N., Sinor, P. N., Pitkar, V. K., Murti, P. R., Irani, R. R., Shak, H. T., Kadam, P. M., Iyer, K. S. S., Iyer, M. M., Hedge, A. K., Chandrashekar, G. K., Schroff, B. C., Sahiar, B. E. & Mehta, M. N.: Incidence rates of oral cancer and natural history of oral precancerous lesions in a 10-year follow-up study of Indian villagers. Community Dent Oral Epidemiol *8:* 287, 1980.
4. Holmstrup, P., Dabelsteen, E. & Roed-Petersen, B.: Oral leukoplakia transplanted to nude mice. Scand J Dent Res. In press.
5. Pindborg, J. J., Jølst, O., Renstrup, G. & Roed-Petersen, B.: Studies in oral leukoplakia: A preliminary report on the period prevalence of malignant transformation in leukoplakia based on a follow-up study of 248 patients. J Am Dent Assoc *76:* 767, 1968.
6. Roed-Petersen, B. & Renstrup, G.: A topographical classification of the oral mucosa suitable for electronic data processing. Its application to 560 leukoplakias. Acta Odontol Scand *27:* 681, 1969.
7. Roed-Petersen, B., Gupta, P. C., Pindborg, J. J. & Singh, B.: Association between oral leukoplakia and sex, age and tobacco habits. Bull WHO *47:* 13, 1972.
8. Roed-Petersen, B. & Pindborg, J. J.: A study of Danish snuff-induced oral leukoplakias. J Oral Pathol *2:* 301, 1973.
9. Silverman, S. Jr & Rozen, R. D.: Observations of the clinical characteristics and natural history of oral leukoplakia. J Am Dent Assoc *76:* 772, 1968.
10. Sugár, L. & Bánóczy, J.: Follow-up studies in oral leukoplakia. Bull WHO *41:* 289, 1969.
11. WHO Collaborating Centre For Oral Precancerous Lesions: Definition of leukoplakia and related lesions: An aid to studies on oral precancer. Oral Surg *46:* 518, 1978.

Inhibited Proliferation of Human Scleroderma Skin Fibroblasts and Rheumatoid Synovial Cells with Griseofulvin *in vitro*

G. C. Priestley and J. C. Brown[1]

Department of Dermatology, University of Edinburgh, Edinburgh, Scotland

Received August 7, 1981

Abstract. Griseofulvin at 2–17 μg/ml *in vitro* inhibited the proliferation of scleroderma skin fibroblasts and rheumatoid synovial cells. The inhibition was concentration-dependent, with little difference between the two types of cells. Mean ID_{50} values from the four strains of each group were 9.2 for fibroblasts and 9.5 for synovial cells. The results show that griseofulvin at therapeutic concentrations can have a direct effect on the growth of cells cultured from diseased human connective tissues.

Key words: Griseofulvin; Skin fibroblasts; Scleroderma; Rheumatoid arthritis

We have shown that the antifungal antibiotic griseofulvin inhibits proliferation, glycosaminoglycans (GAG) secretion and protein synthesis in fibroblasts grown from infant foreskin (8). These are characteristic actions of anti-inflammatory drugs, in agreement with previous reports of anti-inflammatory activity for griseofulvin (9), and are of interest in relation to griseofulvin's suggested clinical use in several connective tissue diseases. Because the behaviour of infant genital skin may differ from diseased adult skin on other sites or from other connective tissues, we have now examined the effect of griseofulvin on the proliferation of scleroderma skin fibroblasts and rheumatoid synovial cells in culture.

METHODS

Derivation of four strains of scleroderma skin fibroblasts (SD 3, 6, 9 and 10) and four strains of rheumatoid synovial cells (RA 1, 2, 5 and 6) has been reported elsewhere (7). Cultures were maintained at 37°C in Dulbecco-Eagle medium containing 10% foetal calf serum, 4 mm glutamine, 100 units/ml penicillin and 100 μg/ml streptomycin (all from Gibco-Europe, Paisley, Scotland) with refeeding on alternate days. Cells were in passages 6–12.

[1] *Present address:* Inveresk Research International, Invereskgate, Musielburgh, EH21 7UB, Scotland.

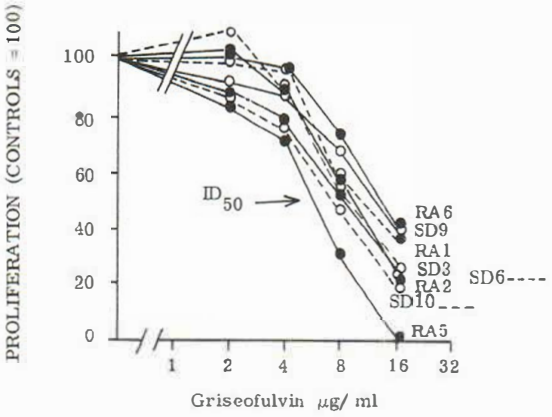

Fig. 1. Inhibition of proliferation by griseofulvin: effects on individual strains of scleroderma fibroblasts (○) and rheumatoid synovial cells (●).

Proliferation tests were performed as described previously (7), using replicate cultures in small flasks (25 cm^2 area) seeded with 10^5 cells. Griseofulvin was dissolved in dimethyl sulphoxide and added to media to give final concentrations of 2, 4, 8 and 17 μg/ml griseofulvin in 0.1% dimethyl sulphoxide. Controls received 0.1% dimethyl sulphoxide only. Griseofulvin was present in the cultures from the third to the sixth day, each concentration of the drug being tested on four (occasionally three) cultures. Cell counts were performed on control cultures on day 3 and on control and treated cultures on day 6, and the increase in cell number over days 3 to 6 was expressed as a percentage of that in controls. Counts were made on cell suspensions prepared with trypsin–versene, using a Coulter counter. Griseofulvin concentrations giving 50% inhibition (ID_{50}) were read from semilogarithmic plots of proliferation versus concentration (see Fig. 1).

RESULTS

Dose–response curves for individual cell strains are shown in Fig. 1. In keeping with previous experience, individual strains within each group varied in their response. ID_{50} values ranging from 6–13 μg/ml. Griseofulvin at 17 μg/ml produced 59–99% inhibition. Proliferation of one strain (SD 6) was significantly stimulated at 2 μg/ml (controls 100±1 (S.E.M.) drug-treated 110±2; $p<0.02$). The overall effects in the two groups of cell strains were remarkably similar (Fig. 2) with mean ID_{50} values of 9.2 and 0.5 μg/ml.

Rheumatoid synovial cells grow in rather disorganized swirling patterns on plastic surfaces, compared with fibroblasts, and griseofulvin at 2 μg/ml appeared to improve the regularity of the swirling without notably affecting proliferation, which was 98, 103, 84 and 101% of controls in the four strains at this concentration.

DISCUSSION

Fibroblast cultures have proved a useful test system for various drugs, but use of non-human or otherwise inappropriate cell strains has limited the clinical interest of the data. These results show that griseofulvin can have a direct effect on the proliferation of cells cultured from diseased human connective tissue. The inhibition recorded resembles that found in previous work with other cell strains (8), where effects on GAG secretion and total protein synthesis were also encountered. In tests on fibroblasts *in vitro* GAG secretion is even more susceptible to inhibition by drugs than is cell proliferation, and is inhibited at lower drug concentrations, while protein synthesis is more resistant, only being affected at drug concentrations causing appreciable (>50%) inhibition of proliferation (7, 8). The fact that proliferation begins to be affected at griseofulvin concentrations (2–4 μg/ml) resembling peak serum values in patients receiving oral therapy (6) suggests that similar modulation of connective tissue cell behaviour may occur in vivo. Benefits from griseofulvin have been reported for patients with shoulder/hand syndrome (2), scleroderma (3) and Raynaud's phenomenon (1).

The mechanism of griseofulvin's effect on cell proliferation is uncertain. In a range of fungal and mammalian cells it impairs microtubule function, producing metaphase arrest of mitosis or the ap-

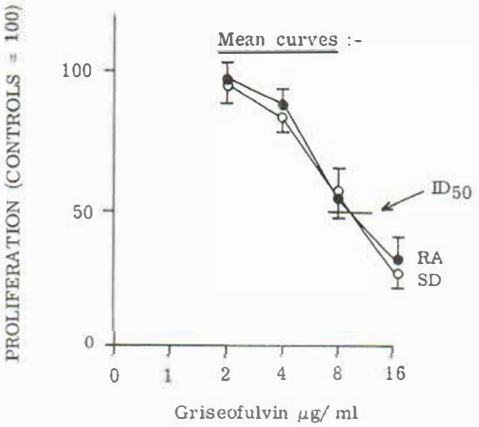

Fig. 2. Inhibition of proliferation by griseofulvin: composite curves for scleroderma fibroblast strains (○) and synovial strains (●).

pearance of multinucleate cells (4, 10) but we did not see these effects in human skin fibroblasts (8). Many actions of anti-inflammatory drugs are mediated through inhibition of prostaglandin synthesis or availability and griseofulvin can block the effects of prostaglandin E_2 (9). Dose–response curves for prostaglandins tend to follow a characteristic bell-shape with opposite effects at high and low concentrations (5). Part of such a curve might be apparent in Fig. 1 if prostaglandin was responsible for the effects on cell proliferation. In fact only one cell strain showed stimulated proliferation at low griseofulvin concentration. Other drugs with anti-inflammatory properties have given similarly inconsistent low-dose stimulation in our previous work (7), so the evidence for prostaglandin involvement is not convincing.

ACKNOWLEDGEMENT

This work was supported by a grant from the Medical Research Council.

REFERENCES

1. Allen, B. R.: Griseofulvin in Raynaud's phenomenon. Lancet *ii:* 840, 1971.
2. Cohen, A., Goldman, J., Daniels, R. & Kanenson, W.: Treatment of shoulder–hand syndrome with griseofulvin. JAMA *173:* 542, 1960.
3. Giordano, M.: Griseofulvin for scleroderma. Lancet *ii:* 260, 1967.
4. Gull, K. & Trinci, A. P. J.: Griseofulvin inhibits fungal mitosis. Nature *244:* 292, 1973.
5. Horobin, D. F.: Interactions between prostaglandins and calcium: the importance of bell-shaped dose response curves. Prostaglandins *14:* 667, 1977.
6. Platt, D. S.: Plasma concentrations of griseofulvin in human volunteers. Br J Dermatol *83:* 382, 1970.
7. Priestley, G. C.: Changes in the growth and metabolism of cells cultured from normal, sclerotic and rheumatoid connective tissue brought about by D-penicillamine and by sodium salicylate. J Invest Dermatol *74:* 413, 1980.
8. Priestley, G. C. & Brown, J. C.: Effects of griseofulvin on the morphology, growth and metabolism of fibroblasts in culture. Br J Dermatol *99:* 245, 1978.
9. Sorrentino, L., Capasso, F. & Di Rosa, M.: Anti-inflammatory properties of griseofulvin. Agents Actions *7:* 157, 1977.
10. Weber, K., Wehland, J. & Herzog, W.: Griseofulvin interacts with microtubules both in vivo and in vitro. J Mol Biol *102:* 817, 1976.

Exanthema fixum Due to Ultraviolet Radiation

J. Langeland

Department of Dermatology, Rikshospitalet, Oslo, Norway

Received August 17, 1981

Abstract. A case of exanthema fixum caused by sun exposure and reproducible by UV-A and UV-B exposure tests is described.

Key words. Exanthema fixum; Photosensitivity; Ultraviolet radiation

The concept of exanthema fixum was introduced by Brocq, who in 1894 described a rash consisting of sharply demarcated round or ovoid edematous plaque caused by phenazone (1). The number of drugs known to be capable of producing this characteristic lesion is now large and still increasing (2, 5, 6). Various foods as possible causes have also been mentioned (3). However, as far as is known, there is no report among existing literature of fixed eruptions having been caused by electromagnetic waves.

CASE REPORT

A 47-year-old man was referred to our clinic with a rash which had occurred after sun exposure of 30 min at about 60°N latitude (Oslo). The man was not taking medicaments of any kind. He had experienced an identical eruption each time he had stayed in the sun for a certain time over the last four summers. The lesion appeared during or after the exposure, itched and disappeared in a couple of weeks, leaving slightly pigmented areas. There were partly detached, partly confluescent ovoid plaques 2–6 cm in diameter with erythema, pigmentation and a sharp demarcation from normal skin on the patients's legs (Fig. 1). Histological examination showed superficial and deep subacute inflammation, not specific for, but consistent with the diagnosis of exanthema fixum (4).

MATERIALS AND METHODS

After the rash had disappeared, light tests were performed. For the UV-B test a Xenon Arc Lamp with a Schott WG 295 cut-off filter was used. This gives a continous spectrum of electromagnetic waves above 295 nm. For the UV-A test a Waldmann UV 800 with Sylvania