

## Proliferative Responses of Fibroblasts from Psoriatic and Normal Skin to Clobetasol Propionate

G. C. PRIESTLEY, L. W. ADAMS, C. A. OAKLEY and R. J. PRESCOTT<sup>1</sup>

*Department of Dermatology, and <sup>1</sup> Medical Computing and Statistics Unit, University of Edinburgh, The Royal Infirmary, Edinburgh EH3 9YW, Scotland*

Priestley G C, Adams L W, Oakley CA, Prescott R J. Proliferative responses of fibroblasts from psoriatic and normal skin to clobetasol propionate. *Acta Derm Venereol (Stockh)* 1983; 63: 393-396.

The proliferation of fibroblasts cultured from psoriatic and normal skin was compared following addition of clobetasol propionate to the culture medium. Proliferation was stimulated at 0.1 µg/ml and progressively inhibited at 1-10 µg/ml. A differential sensitivity to the drug was demonstrated: the fibroblasts from involved psoriatic skin were more stimulated than normal fibroblasts at 0.1 µg/ml and then more inhibited at 10 µg/ml. The fibroblasts from uninvolved psoriatic skin displayed intermediate responses. There was some cytotoxicity in cultures of the psoriatic cells at 10 µg/ml. The results demonstrate a further abnormality of fibroblasts from psoriatic skin. *Key words: Psoriasis; Fibroblasts; Clobetasol propionate.* (Received December 16, 1982)

G. C. Priestley, Department of Dermatology, The Royal Infirmary, Edinburgh EH3 9YW, Scotland.

We have reported that fibroblasts cultured from the involved and uninvolved skin of patients with severe plaque psoriasis proliferate faster and synthesize more collagen and other protein than fibroblasts from comparable sites on control subjects (5, 6). The psoriatic fibroblasts are also abnormally dependent on serum in the culture medium for anchorage and assume a rounded form in serum-free medium. In this paper we report a further abnormality of the psoriatic fibroblasts—an increased sensitivity to the corticosteroid clobetasol propionate.

### METHODS

Fibroblast strains isolated from the involved (PSA strains) and uninvolved (PSB strains) forearm skin of male patients with severe plaque psoriasis, as previously reported (6), were stored in liquid nitrogen until required. Fibroblasts derived from the forearms of normal male subjects (NSF strains) were also available (6). Four strains of each type at the 4th-6th passage were used in this work. Average ages of the donors were PSA 46, PSB 40, NSF 39 years.

The cells were grown in Dulbecco-Eagle medium with 4mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin and 10% foetal calf serum (all from Gibco Europe, Paisley, Scotland). Newly seeded cultures were flushed with 5% CO<sub>2</sub>/95% air before incubation at 37°C and medium was changed three times weekly.

Clobetasol propionate was dissolved in 1:1 dimethyl sulphoxide-propylene glycol for addition to culture media. Appropriate dilutions were made to give 0.1, 1, 5 and 10 µg/ml. The steroid was donated by Glaxo Laboratories Limited and was shown by paper chromatography to be at least 99.5% pure.

Proliferation experiments were carried out as in previous work (3,6,7). To test four concentrations of drug, 24 replicate cultures were established in 24-well plastic dishes (growth area of each well 2 cm<sup>2</sup>), using 10<sup>4</sup> cells per well (Day 0). Four wells were assigned to each concentration of drug, with two control groups. On Day 1 all media were replaced. On Day 3 one control group was used for cell counts: the cells were released with trypsin-versene and counted electronically in a Coulter Counter. In some experiments viability counts were performed in a haemocytometer using a 0.5% solution of Trypan Blue. Other wells on Day 3 received media with the appropriate concentration of drug. All media were replaced on Day 4, and on Day 6 cell counts were made on all the wells. The proliferation

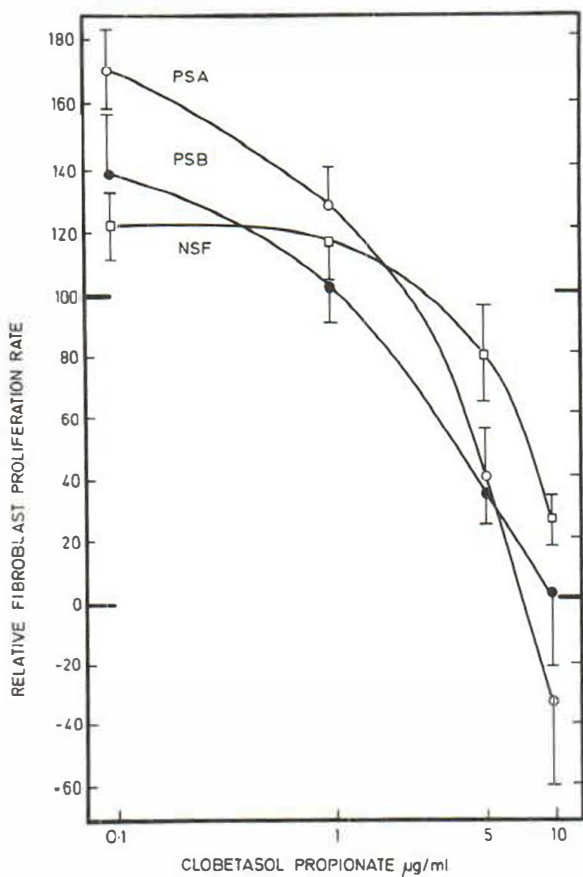


Fig. 1. Effect of clobetasol propionate on relative proliferation rates of psoriatic and normal skin fibroblasts. Composite dose-response curves were compiled from the data for four strains of each type with four cultures of each strain at each drug concentration; values shown are means  $\pm$  SE. The proliferation of untreated cultures of each strain is represented as 100; values exceeding 100 show stimulation by the drug, values below 100 indicate inhibition. PSA fibroblasts (○) are from involved psoriatic skin, PSB fibroblasts (●) from uninvolved psoriatic skin and NSF fibroblasts (□) from normal skin.

rate of cells in drug-treated cultures was expressed as a percentage of the corresponding proliferation rate of untreated controls over Days 3-6.

As a further test of the viability of steroid-treated fibroblasts, recovery experiments were performed in which cultures treated with 10  $\mu$ g/ml clobetasol propionate for Days 3-6 were allowed to continue their growth for a further 4 days, with or without the steroid.

Statistical analysis of the data was performed on relative proliferation rates. Absolute proliferation rates were first derived as  $\log n_6 - \log n_3$ , where  $n_6$  is the mean cell count on Day 6 for a particular treatment group and  $n_3$  is the control cell count on Day 3. If  $K_0$  represents the absolute proliferation of control cultures and  $K_{0.1}$ ,  $K_1$  etc. the proliferation rates of treated cultures, then  $K_{0.1}/K_0$  is the relative proliferation rate.

An analysis of variance was performed on the full set of relative proliferation rates to test for differences in dose response (the data conforms to a 'split-plot' design and the interaction between treatment group and dose provides a test for differences in the dose-response curves (11)). To reinforce the conclusions without the need for assumptions of normal distributions, the data for the 0.1  $\mu$ g/ml and 10  $\mu$ g/ml concentrations were each analysed using the following non-parametric technique. If we consider involved psoriatic, uninvolved psoriatic, and normal skin as having a natural ranking in terms of abnormality which also applies to the proliferative capacity of the fibroblast strains PSA, PSB and NSF (6), then we may calculate the Spearman rank correlation ( $R$ ) to determine whether there is a statistically significant association between the abnormality grouping of the strain and the relative fibroblast proliferation rate.

## RESULTS

Composite dose-response curves from the four fibroblast strains in each group are shown in Fig. 1. The general effect was a variable stimulation of proliferation at 0.1  $\mu$ g/ml and

progressive inhibition at 1–10 µg/ml, so that at 10 µg/ml proliferation was virtually halted in most strains. A differential sensitivity to the drug was also apparent at the highest and lowest concentrations. This was demonstrated in two ways. The analysis of variance showed that there was a statistically significant interaction between the type of strain and the steroid concentration ( $F=2.6$ ;  $p<0.05$ ), demonstrating overall differences in the three dose–response curves. The non-parametric method showed that at 0.1 µg/ml the rate of cell proliferation increased in order from the NSF to the PSB to the PSA fibroblast strains ( $R=+0.62$ ;  $p<0.05$ ). In contrast, at the 10 µg/ml level there were differences in the cell proliferation rates in the opposite direction ( $R=-0.65$ ;  $p<0.05$ ).

At 10 µg/ml, three of the four PSA strains and one PSB strain had fewer cells than on Day 3, suggesting that the effect of the drug was not merely cytostatic but also cytotoxic. Although the normal fibroblasts increased in number, they were still severely inhibited in comparison with their untreated controls. At the intermediate concentrations there were no clear differences between the strains.

In two experiments in which PSA fibroblasts were treated with 10 µg/ml steroid, viabilities averaged 48% and 65% in the treated cultures on Day 6, vs. 97% in untreated PSA controls, thus confirming that many cells had been killed. However, in recovery experiments where the cultures treated with 10 µg/ml clobetasol propionate were continued after Day 6, the cell totals increased beyond the Day 6 control values, and the presence or absence of steroid from Days 6–10 made little difference.

## DISCUSSION

These data show that fibroblasts from psoriatic skin are not only hyperactive (5, 6) but also hyper-reactive. We have studied a single, very potent, corticosteroid, but it seems unlikely that the increased sensitivity of the cells is specific to this one compound. A differential response to hydrocortisone has been seen in fibroblasts from normal and keloid tissue (10), and in skin fibroblasts from normal and diabetic subjects (8). In contrast, fibroblasts from normal and scleroderma skin had similar reactions to corticosteroids, D-penicillamine and sodium salicylate (4, 7). The reactions of psoriatic fibroblasts to drugs have not been studied before.

To our knowledge, stimulation of proliferation by clobetasol propionate has not been reported previously, although we and others have noted some modest stimulation of normal skin fibroblasts with several other corticosteroids (1, 3, 7, 9). In extended dose–response experiments even 0.001 µg/ml clobetasol propionate (results not shown) stimulated the proliferation of PSA fibroblasts.

The inhibitory effect of the corticosteroid described here is mainly cytostatic, but at 10 µg/ml there was some cytotoxicity in the psoriatic fibroblasts. It was also clear from viability tests and recovery experiments that the cells surviving an initial toxicity were capable of further proliferation even in the presence of the drug; these observations confirm some of the findings of Ponec et al. (2) with clobetasol propionate. It is not known whether the dead cells represent a random fraction, or those at a particular stage of the cell cycle, or whether they have other distinctive characteristics.

## REFERENCES

1. Ponec M, Hasper I, Vianden G D N E, Bachra B. Effects of glucocorticosteroids on primary human skin fibroblasts. II. Effects on total protein and collagen biosynthesis by confluent cell cultures. *Arch Dermatol Res* 1977; 259: 125.
2. Ponec M, de Haas C, Bachra B N, Polano M K. Effects of glucocorticosteroids on cultured

- human skin fibroblasts. III. Transient inhibition of cell proliferation in the early growth stages and reduced susceptibility in later growth stages. *Arch Dermatol Res* 1979; 265: 219.
3. Priestley G C. Effects of corticosteroids on the growth and metabolism of fibroblasts cultured from human skin. *Br J Dermatol* 1978; 99: 253.
  4. 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* 1980; 74: 413.
  5. Priestley G C. Hyperactivity of fibroblasts cultured from psoriatic skin. II. Synthesis of macromolecules. *Br J Dermatol* 1983: 109.
  6. Priestley G C, Adams L W. Hyperactivity of fibroblasts cultured from psoriatic skin. I. Faster proliferation and effect of serum withdrawal. *Br J Dermatol* 1983: 109.
  7. Priestley G C, Brown J C. Effects of corticosteroids on the proliferation of normal and abnormal human connective tissue cells. *Br J Dermatol* 1980; 102: 35.
  8. Rowe D W, Starman B J, Fujimoto W Y, Williams R H. Abnormalities in proliferation and protein synthesis in skin fibroblast cultures from patients with diabetes mellitus. *Diabetes* 1977; 26: 284.
  9. Runikis J D, McLean D, Stewart W D. Growth rate of cultured human fibroblasts increased by glucocorticosteroids. *J Invest Dermatol* 1978; 70: 348.
  10. Russell J D, Russell S B, Trupin K M. Differential effects of hydrocortisone on both growth and collagen metabolism of human fibroblasts from normal and keloid tissue. *J Cell Physiol* 1978; 97: 221.
  11. Snedecor G W, Cochran W G. *Statistical Methods*. 6th ed. Iowa State University Press. 1967: 370-373.