The Effect of Calcipotriol on Lesional Fibroblasts from Patients with Active Morphoea

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We examined the responsiveness of cultured dermal fibroblasts from biopsies of 6 patients with active morphoea and a similar number of matched controls to the cell proliferation inhibition activity of calcipotriol. Cultured fibroblasts from controls showed no significant response to calcipotriol (at concentrations of 1 x 10⁻⁶ to 1 x 10⁻⁴ M). However, calcipotriol did inhibit the proliferation response of morphoea fibroblasts at all concentrations when compared with controls. There was 4- to 20-fold inhibition in 2 of the morphoea patients when compared with control samples. Four other morphoea samples showed inhibition but to a lesser extent compared with controls. Key words: localised scleroderma; vitamin D₃.

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Histological examination of involved morphoea skin shows increased dermal collagen. This is likely to be due to a clonal overactivity of lesional fibroblasts. Direct in situ hybridizations of morphoea skin have shown a subpopulation of activated fibroblasts in the papillary and upper reticular dermis, with increased levels of procollagen mRNA (1). When the same group cultured lesional fibroblasts from patients with morphoea, increased levels of procollagen mRNA were demonstrated in some (2/6 patients) of the cell lines (2). However, no evidence of a subpopulation responsible for elevated collagen production was found using cytoplasmic dot hybridization, suggesting that “activated” fibroblasts may be selected in culture. Similar findings have been reported in systemic sclerosis, where a subpopulation of high collagen producing fibroblasts which appear to be located in the papillary and upper reticular dermis (3). Normal human dermal fibroblasts have been shown to possess receptors for calcipotriol (4, 5). We report on the response to calcipotriol shown by cultured fibroblasts from healthy subjects and patients with morphoea.

MATERIALS AND METHODS
Volunteers with histologically proven morphoea were selected. In all cases the morphoea lesion was still expanding and had an active inflammatory margin. Biopsies were taken from the inner aspect of the inflammatory edge of 5 women and one man, aged between 14 and 57 years. The lesions varied in size from 8 to 60 cm². They were compared with 5 age- and sex-matched controls who had undergone removal of benign pigmented lesions from the same region as their matched patient.

Primary cultures of fibroblasts were established in the usual manner. Fibroblasts were grown in monolayer cultures and maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% newborn calf serum (NBCS). When ready, cells were seeded at 3 x 10⁵ cells per well in 96 well plates and cultured for 24 h. The medium was changed to DMEM containing 2% NBCS and varying concentrations of between 10⁻⁶ to 10⁻⁴ M calcipotriol or ethanol vehicle (0.01%). The calcipotriol was obtained from Leo Pharmaceuticals. Cells were used at passage 4 to 6. All assays were performed in triplicate. The rate of cell proliferation was measured using the MTT assay (6). This assay is based on the reduction of the soluble yellow MTT tetrazolium salt to the insoluble blue MTT formazan. This reaction is driven by mitochondrial succinic dehydrogenase and allows accurate measurement of cell proliferation. The absorbency was determined spectrophotometrically at 570 nm using an ELISA reader and the number of viable cells calculated by reference to a standard curve of optical density. Both the morphoea and control fibroblasts were examined simultaneously during the same procedures, which remained constant. Results were corrected for controls.

RESULTS
The results presented are means of 1–3 assays performed in triplicate. Calcipotriol had significant effects on the extent of

![Figure 1](image-url)  
**Fig. 1.** MTT proliferation assay of morphoea and normal fibroblasts. Each point represents mean ± SEM. *p < 0.01. ☻: Morphoea; △: Normals.
morphoea fibroblast proliferation in monolayer cultures (Fig. 1) at all concentrations when compared with controls. Statistical analyses were performed using Wilcoxon unpaired rank sum test. There was 4-20-fold inhibition in 2 of the morphea patients when compared with control samples. Four other morphea samples showed inhibition but to a lesser extent compared with controls (Fig. 2 shows individual patient responses to calcipotriol). The two most responsive morphea fibroblast lines were from a 14-year-old female patient with a 3-year history of neck involvement and a 50-year-old female with morphea involving the abdomen for 4 years.

**DISCUSSION**

Calcipotriol is a drug with many properties; these may include the ability to alter fibroblast collagen and fibronectin synthesis as well as to inhibit fibroblast proliferation. As the primary abnormality in morphea is likely to be related to abnormal fibroblast proliferation and/or collagen or matrix protein production from these fibroblasts we chose to firstly look at the in vitro response of morphea fibroblasts to calcipotriol. This compound now has the value of being easily available for topical use in the clinical setting. There have been relatively few studies on the in vitro effect of vitamin D3 on human fibroblasts, but those that have been published appear to show a dose-dependent inhibition of proliferation to 1,25-dihydroxyvitamin D3 (calcitriol) (4, 5). The concentrations of calcitriol used in these studies varied from $10^{-10}$ to $10^{-8}\text{M}$; unfortunately only single patient results were reported in both cases. This was confirmed by the study of Dobak et al. (7), showing suppression of fibroblast proliferation in a culture of neonatal foreskin fibroblasts after exposure to $10^{-8}\text{M}$ 1,25-dihydroxyvitamin D3, this was accompanied by an increased collagen production. This contrasts with a study using rabbit marrow fibroblasts which failed to show any inhibition of proliferation with calcipotriol and in which a significant reduction in collagen production was observed (8).

In this study only patients with active morphea, as suggested by increasing size of lesion and violaceous edge, were selected. All patients had histologically confirmed diagnoses. All the biopsies for culture were taken from the inner aspect of the active edge, so as to ensure that the fibroblast population was likely to be active and so amenable to influence of calcipotriol. The results of this experiment show that calcipotriol had significant effects on the extent of morphea fibroblast proliferation in monolayer cultures at all concentrations when compared with controls. In 2 of the morphea patients there was 4- to 20-fold inhibition when compared with control samples. The control patient's fibroblasts showed no inhibition of proliferation when exposed to calcipotriol.

These results also suggest that our original supposition that calcipotriol would cause a pronounced inhibition of normal human fibroblast proliferation in vitro may have been incorrect. The different response of the morphea fibroblasts in this study may be due to an increased sensitivity of the vitamin D3 receptors expressed by the morphea fibroblasts, leading to the inhibition of proliferation seen. The range of response of the morphea fibroblasts suggests there may be a variable sensitivity to calcipotriol, which could translate into a variable clinical response of patients to this compound, and it would have been interesting to measure vitamin D3 receptor levels on these fibroblasts, but unfortunately we were unable to do this.

These in vitro results give grounds for optimism that a vitamin D3 analogue might prove therapeutically useful in treatment of morphea for some if not all patients. Thus far, vitamin D3 therapy in morphea has been confined to two reports. Humbert et al. (9) report the case of a woman with a 2-year history of disseminated morphea who improved after receiving 0.5 µg oral 1,25-dihydroxyvitamin D3 (calcitriol) for 4 months. Hulshof et al. report an improvement in 3 patients with generalized morphea after 3 to 7 months' treatment with oral daily calcitriol (10). There is also a report of the use of high-dose oral 1,25-dihydroxyvitamin D3 in 11 patients with systemic sclerosis showing improvement in skin changes, as compared with baseline values over a period of between 12 months to 3 years (11). In view of the in vitro results in this study and previous results with high-dose oral vitamin D3, topical therapy with vitamin D3 may be of therapeutic benefit. Penetration of topical 1,25-(OH)2-D3 into the dermis might be a problem, however; studies with topical 1,25-(OH)2-D3 in patients with psoriasis showed a systemic absorption of about 2% of the compound, suggesting that there must be dermal penetration of topical 1,25-(OH)2-D3. We therefore think that the possible use of calcipotriol in the management of morphea needs further investigation.

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REFERENCES


