Immunomodulation of Psoriasis with a Topical Cyclosporin A Formulation

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Topical cyclosporin A (CyA; Sandimmun) in a formulation incorporating the penetration enhancers (PE) propylene glycol (18%) and azone (2%) was tested for efficacy in a double-blind, vehicle-controlled trial in 5 chronic plaque psoriatic patients. On each patient, two similar plaques were treated daily, under occlusion, for 4 weeks with either 8% (w/v) CyA, containing PE, or with vehicle comprising olive oil with PE. All sites improved significantly, but there was no significant difference between those receiving active and control preparations. Cryostat sections of biopsies performed after 4 weeks' treatment showed significant reductions in CyA compared with vehicle-treated sites in the number of cells, positive for CD3 and CD25 in the epidermis and CD25 and HLA-DR in the dermis. These results suggest that amounts of CyA adequate to affect the lymphocytic infiltrate penetrated the epidermis but that only partial suppression occurred in the dermis, as indicated by the reduction in lymphocyte activation status. *Key words: Lymphocytes; Activation antigens; Adhesion molecules.*

The selective anti-T helper lymphocyte properties of the immunosuppressant drug cyclosporin A (CyA) have encouraged its clinical use in the treatment of dermatoses with a presumed T cell-mediated pathogenesis, although nephrotoxicity and hypertension, induced during long-term treatment, have limited its use to more severely affected patients (1). Topical application of CyA may circumvent such toxicity; however, little or no clinical efficacy with topical CyA at concentrations of 0.1–10% (w/v) in oil or unguentum Merck vehicles has been demonstrated in either allergic contact sensitivity (2, 3) alopecia areata (4, 5) or psoriasis (6–8). In contrast, intradermal injections of CyA into psoriatic lesions have been highly successful in clearing psoriatic plaques and in significantly reducing the numbers of infiltrating mononuclear cells (9, 10). Furthermore, when CyA was applied as a mouth wash to the non-keratinised mucosal surface of patients with oral lichen planus, clearing resulted in almost all patients (11), suggesting that the lack of a therapeutic response with topical CyA in skin is probably due to inadequate drug penetration across the stratum corneum, the principal barrier to percutaneous drug absorption.

We have previously shown that the penetration enhancers (PE) azone and propylene glycol enhance CyA penetration a hundredfold through human stratum corneum in vitro and that in guinea pigs undergoing delayed-type hypersensitivity reactions, CyA and PE significantly reduced the number of infiltrating lymphocytes in reaction sites compared with CyA alone (12). On the basis of these findings we conducted a double-blind, vehicle-controlled study to determine the clinical and immunological efficacy of this topical formulation in psoriasis.

MATERIALS AND METHODS

Drugs

A CyA solution at a final concentration of 8% w/v, and containing PE, was made from the 10% w/v oral preparation of CyA (Sandimmun, Sandoz Ltd, Basle, Switzerland). Sandimmun was diluted with olive oil (Thornton and Ross, Huddersfield, UK) and contained the PE azone (Whitby Research, Richmond, Virginia, USA) at 2% v/v and propylene glycol (Sigma, Poole, Dorset, UK) at 18% v/v. The vehicle solution consisted of olive oil and the appropriate concentrations of both PE.

Patients and treatment

Five male patients, aged 33–68 years, with chronic plaque psoriasis had two equivalent lesions on the trunk or arms treated once daily with either topical CyA or vehicle for 4 weeks. Each coded solution was applied thinly and evenly to respective lesions, which were then occluded with polythene dressings. Informed consent was obtained from each patient and the study was approved by the local Ethics Committee.

Assessment of disease activity

The clinical appearance of lesions prior to and after treatment was assessed 'blind' by visually scoring from 0–4 for severity with respect to each of the following: erythema, thickness and scaling. The maximum possible score was 12. Erythema was also measured by photo reflectance, using an erythema meter (Cutech Instruments, High Wycombe, Bucks, UK) on plaques previously moistened with water to prevent optical disturbance from scales. Skin biopsies (3 mm) were taken from the lesions, under local anaesthetic (Lignocaine), 1 cm within the lesion edge and mounted in OCT medium (Miles, Stoke Poges, UK) and snap-frozen in arcton/liquid nitrogen, then stored at −20°C prior to sectioning. No biopsies were taken prior to treatment as this would breach the barrier to penetration and influence the results.

Immunohistochemistry

Cryostat sections (6 μm) were fixed in acetone for 20 min, air-dried, then incubated for 1 h at room temperature with the following primary mouse anti-human monoclonal antibodies diluted to their appropriate concentration in 0.005 M Tris-buffered saline (TBS), pH 7.6, T6 (CD1, Langerhans' cells) 1:10, T4 (CD4, T helper lymphocytes, Langerhans' cells) 1:10, LFA-1 (CD11a, T, B cells, monocytes, granulocytes) 1:50, Il-2R (CD25, activated T,B cells, macrophages) 1:25, BRC (CD14, tissue macrophages) 1:500, Dako, High Wycombe, UK; Leu4 (CD3, Pan T cells) 1:40, Leu11b (CD16, granulocytes, NK cells) 1:20, Becton Dickinson, Oxford, UK; T8 (CD8, T suppressor lymphocytes) 1: 20, HLA-DR (Langerhans' cells, macrophages, monocytes, B cells, activated T cells) 1:40, SAPU, Carluke, UK; ICAM-1 (CD54,

Table I. *Clinical response to topical CyA or vehicle treatment*

Results are means ± 1SD. Symbols represent statistically significant differences from pre-treatment values.

Week	Visual score		Erythema meter reading	
	Vehicle	CyA	Vehicle	CyA
0	8.0 ±1.2	8.2 ±1.3	42.6 ±5.7	42.8±9.0
4	3.6*±1.8	3.3*±2.0	33.2+±3.6	33.4±7.9

*$p < 0.02$; +$p < 0.05$.

monocytes, macrophages, keratinocytes) 1:200, Dr T Springer. Control slides were incubated with TBS instead of primary antibody. Secondary biotinylated polyclonal rabbit anti-mouse Ig (Dako) diluted 1:200 with 4% v/v normal human serum in TBS was incubated on sections for 30 min. Thereafter, sections were treated for 30 min with streptavidin-biotin complex peroxidase diluted in 0.05 M TBS and the colour was developed by incubation for 6 min with 30 mg diaminobenzidine (Sigma) in 100 ml of 0.05 M TBS containing 20 μl H_2O_2 (Sigma).

Assessment of cellular infiltrate

Cell counts in the epidermis and upper papillary dermis were performed with a ×10 eyepiece and ×25 objective using a point count method and an eyepiece grid occupying about 50% of the field of view. The entire length of each section was examined (approximately 5 fields of view) and cell counts were expressed per mm^2.

Statistics

Results are expressed as the mean ±1 standard deviation. The significance of differences between CyA and vehicle-treated lesions was determined using a paired *t*-test.

RESULTS

Clinical response

Compared with pretreatment values, significant improvements in the external appearance of lesions occurred after 4 weeks, with both CyA and vehicle as assessed visually but only with the vehicle when measured by the erythema meter (Table I). There was no significant difference, by either method of assessment, between the control and CyA-treated lesions.
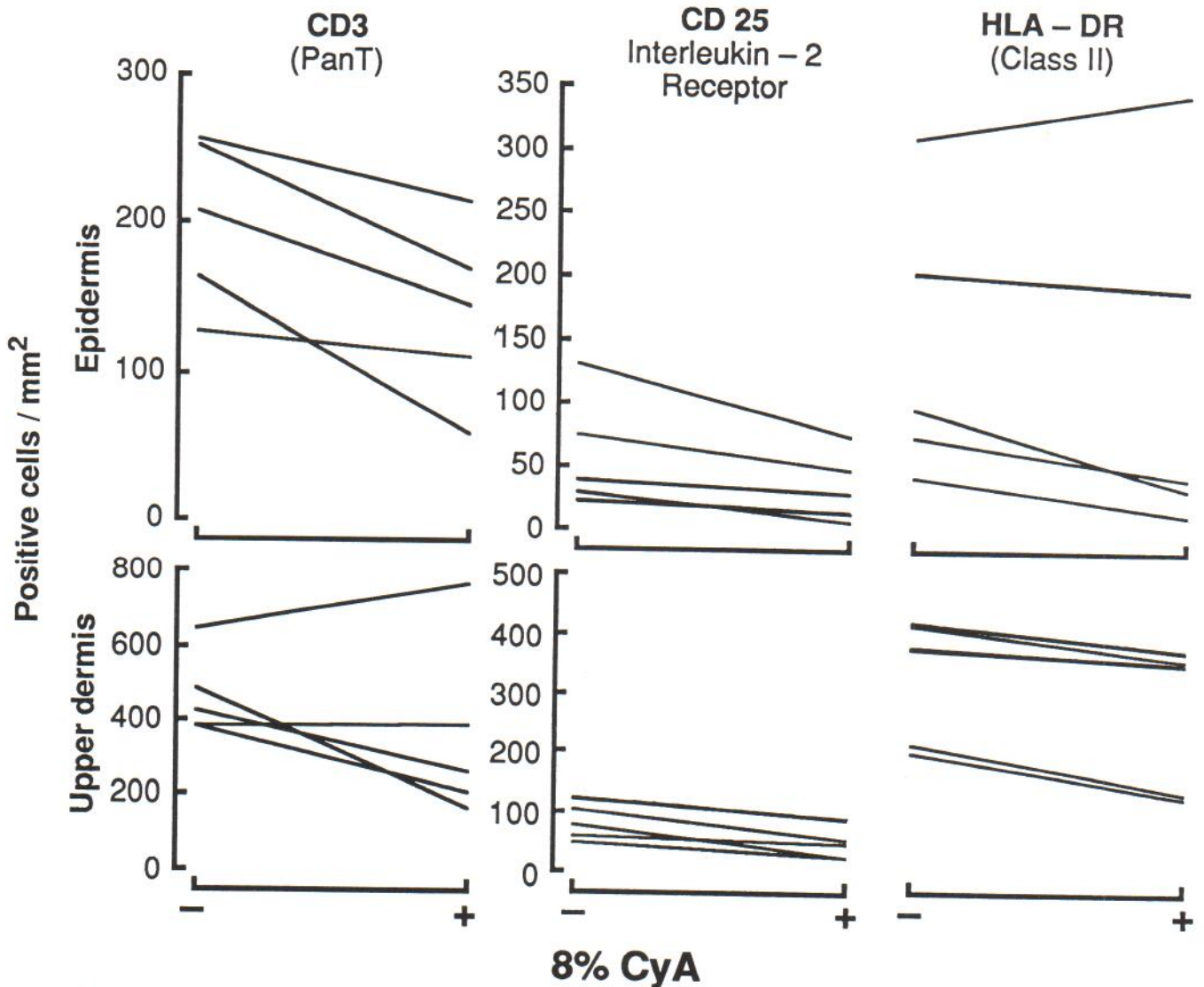

Fig. 1. Effect of topical CyA (+) and vehicle (−) on the number of CD3+, CD25+ and HLA-DR+ cells in the epidermis and dermis of lesional psoriatic skin in 5 patients.

Table II. *The effect of topical CyA on the leucocytic infiltrate in psoriatic lesions*

Results are means ± 1SD, n = 5. Symbols represent statistically significant differences from vehicle-treated lesions.

| | CyA | Number of positive cells/mm² | | | | | | | |
		CD3	CD4	CD8	CD25	HLA-DR	CD1	CD14	CD16
Epidermis	−	202 ±51	138±52	117±36	60 ±40	146 ±98	212±71	23±25	39±27
	+	141*±52	82±26	65±22	34*±24	125 ±127	192±73	61±71	63±57
Dermis	−	462 ±97	268±75	134±35	83 ±27	324 ±98	82±49	9±5	25±17
	+	359 ±216	249±182	121±77	50*±24	268**±112	61±35	45±53	66±51

** p < 0.01, * p < 0.02, ⁺ p < 0.05.

Quantitative immunohistochemistry

CyA-treated lesions had significantly fewer epidermal Pan T (CD3⁺) cells, epidermal and dermal activated (CD25⁺) cells and dermal HLA-DR⁺ cells than those lesions receiving vehicle (Fig. 1 and Table II). The mean combined (epidermis and dermis) percentage reduction in CD3⁺ cells between vehicle and CyA-treated lesions was 28%, while that of CD25⁺ cells was 40%, indicating a substantial reduction in the activation status of the T lymphocytes. There were no significant differences between CyA- and vehicle-treated sites in the number of Langerhans' cells (CD1), tissue macrophages (CD68) or granulocytes and NK cells (CD16) (Table II).

Adhesion molecule expression

The expression of LFA-1 on sections was confined to the infiltrating mononuclear cells in both the epidermis and dermis of vehicle and CyA-treated sites. Overall, the extent of LFA-1 staining was reduced by CyA, which probably results from the associated reduction in lymphocytes. There was some isolated ICAM-1 staining amounting to no more than one or two small areas on epidermal keratinocytes on sections in 4 out of 5 patients from vehicle and CyA-treated sites. The staining was sometimes restricted to the basal keratinocytes but more often also encompassed cells of the malpighian/prickle cell layer. In only one patient did ICAM-1 staining of the keratinocytes disappear with topical CyA, although some infiltrating cells in the epidermis remained positive. Infiltrating monocytes, in both the epidermis and dermis, also expressed ICAM-1, as did the dermal blood vessels in all samples from vehicle- and CyA-treated sites.

DISCUSSION

Recently in an uncontrolled study by Mizoguchi et al., a topical 5% CyA ointment (PASI) induced considerable reductions in disease severity in 10 psoriatic patients following its application to large areas of skin (13). They used a formulation containing ethanol, olive oil, Aerosil 200 (thickening and possible penetration enhancing agent) and polyoxy-5-glyceryl monostearate (non-ionic surface active agent) which differed principally from our formulation only in its viscosity. We also observed a significant improvement in lesional skin with both CyA and the vehicle, which was probably due to the emollient

effect of the solutions and enhanced by constant occlusion, a factor known to improve clinical efficacy (14).

Despite there being no difference between the clinical effect of CyA and its vehicle, skin biopsies revealed that CyA significantly reduced the number of CD3⁺ cells and interleukin-2 receptor (CD25) expressing cells in the epidermis. Although there was no reduction in CD3⁺ cells in the upper dermis of CyA-treated sites, their activation status (CD25 and HLA-DR) was, however, significantly reduced. Compared with vehicle, CyA-treated lesions had 28% fewer CD3⁺ lymphocytes and 40% fewer CD25⁺ cells in the epidermis and dermis, indicating that CyA was principally reducing the number of activated T lymphocytes within these lesions. We have previously shown that successful systemic CyA treatment for one month, which resulted in almost complete resolution of psoriatic plaques, also produced a more pronounced reduction in CD25⁺ cells (60%) than in CD3⁺ cells (48%), and that the accompanying reductions in T lymphocytes were primarily in the dermis (15). It is possible, therefore, that the clearance of inflammatory T cell infiltrates, particularly from the dermis, which occurs during systemic but not topical CyA treatment, is a prerequisite for the resolution of plaques (16–18).

Unlike Schulz et al. (8) who described a reduction only in the number of infiltrating neutrophils in skin treated topically for one week with 5% CyA in an ointment base, we found none even by 4 weeks. In addition, they reported that CyA levels in their treated lesions were equivalent to those in lesions following systemic therapy for one week (8); however, there was no reduction in the number of T lymphocytes or cells expressing activation markers, as has been demonstrated in patients receiving CyA orally for one week (18). Although drug levels were not measured in our skin biopsies, it is probable that CyA was present in sufficiently high concentrations in the epidermis, since the number of CD3⁺ and CD25⁺ cells decreased compared with controls in all patients.

In rats, skin allografts can be maintained with topical CyA (2.5%), but not vehicle, following an initial 10 days of systemic treatment with 8 mg CyA/kg; an initial systemic phase of immune non-responsiveness was critical to the subsequent induction of site-specific immune suppression by topical CyA (19). This could represent an effective and desirable form of therapy for less severely affected psoriasis patients, since systemically-associated drug-induced toxicity could be minimized. In addition, 2 weeks systemic CyA treatment has important immunomodulatory effects in the skin, such as sub-

stantially reducing lesional T lymphocytes (15), antigen-presenting dendritic cells (CD1$^+$, HLA-DR$^+$) (17) and ICAM-1 expression on endothelial cells lining papillary microvessels (20).

In normal skin, the adhesion molecule ICAM-1 is expressed at low frequency on vascular endothelium; however, cytokine-induced upregulation of its expression in sites of inflammation is thought to facilitate the margination and extravasation of T cells bearing the LFA-1 ligand for ICAM-1 (21). In this study there was no loss of ICAM-1 expression on dermal blood vessels, which might explain the continued presence of T cells in the dermis and hence the lack of total resolution of the plaques in these patients.

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