British Journal of Dermatology (1998) **139,** (Suppl. 52), 12–16.

Adapalene 0·1% gel and adapalene 0·1% cream stimulate retinoic acid receptor mediated gene transcription without significant irritative effects in the skin of healthy human volunteers

C.E.M.GRIFFITHS,[1] P.ANCIAN,[2] J.HUMPHRIES, M.PONCET, E.RIZOVA,* S.MICHEL* AND A.CLUCAS*

Section of Dermatology, University of Manchester, Hope Hospital, Salford M6 8HD, Manchester, UK

**Galderma R&D, 635 Rte des Lucioles, BP87, 06902 Sophia-Antipolis, France*

Summary A randomized, investigator masked, intra individual comparative study was conducted in 30 healthy volunteers to compare the cutaneous effects of adapalene 0·1% gel and adapalene 0·1% cream with their respective vehicles, using tretinoin 0·05% cream ($n = 21$) or tretinoin 0·1% cream ($n = 9$) and a tretinoin cream vehicle ($n = 30$) as controls. The products were applied to hip/buttock skin for 4 days under occlusive conditions. Cytosolic retinoic acid binding protein-II (CRABP-II) mRNA levels were measured using the RT-PCR technique in punch biopsies taken from 10 subjects. Epidermal thickness was assessed using image analysis of haematoxylin and eosin stained sections from another 11 subjects. Erythema was assessed in all subjects both by a visual scoring system and by chromameter.

Adapalene 0·1% gel and adapalene 0·1% cream produced similar significant increases in CRABP-II mRNA levels compared to their vehicles ($P < 0·01$). The two tretinoin formulations also resulted in similar significant increases in CRABP-II compared to the cream vehicle ($P < 0·001$). However, only the two tretinoin formulations resulted in an increase in epidermal thickness and only the tretinoin 0·1% cream resulted in significant erythema.

Adapalene 0·1% gel and adapalene 0·1% cream induce RAR-mediated gene expression to a similar degree in this model, without the irritant effects of tretinoin.

Adapalene is a novel naphthoic acid derivative with selective RARβ/γ agonist activity, which modulates cell proliferation and differentiation *in vitro* and shows anti-inflammatory and comedolytic properties *in vivo*.[1] As compared to the standard retinoid agonist, all-*trans*-retinoic acid (referred-to hereafter as tretinoin), adapalene causes significantly less skin irrritation in a variety of clinical pharmacology models[2] and in clinical trials in acne patients.[3] However, it is at least as effective as tretinoin in treating acne,[4] implying that efficacy is not necessarily linked to irritation.

The 4-day occlusion assay has been shown to be a useful model for the evaluation of topical products with retinoid agonist activity. Using this model, retinoic acid receptor (RAR)-mediated events such as the induction of cellular retinoic acid binding protein II (CRABP-II) gene expression[5] can be differentiated from non-specific irritant phenomena such as erythema and epidermal hyperplasia.[6] Indeed, a previous study of adapalene in this model gave results which were entirely consistent with the clinical profile of the molecule: significant induction of CRABP-II mRNA, indicating stimulation of RAR-mediated gene expression, without the irritant effects of tretinoin.[7]

In the 4-day occlusion assay, we compared the effects of two different formulations of adapalene, the previously studied aqueous gel and a new cream which is also well tolerated[8] and effective in the treatment of acne (company data, Galderma, 1998). Tretinoin was used as a positive control.

Correspondence: Dr A.Clucas.

Subjects and methods

This was a randomized, investigator masked, intra individual comparative study conducted in 30 healthy volunteers. The protocol was approved by the Salford and Trafford local ethics committee and each subject provided written informed consent before participating in the study.

Treatment

Each subject received applications of adapalene 0·1% gel (Differin® 0·1% gel, Galderma laboratories, Sophia-Antipolis, France) and adapalene 0·1% cream with their respective vehicle controls. Twenty-one of the subjects also received tretinoin 0·05% cream (Retacnyl® 0·05% cream, Galderma laboratories) and the nine others tretinoin 0·1% cream (Retin-A ® 0·1% cream, Ortho laboratories); all 30 received the vehicle of the tretinoin 0·05% cream as an additional control.

The products were applied to hip/buttock skin for 4 days under occlusive conditions (Opsite® dressing) according to a randomization scheme which ensured that each active product was symmetrically located relative to the corresponding vehicle.

Evaluations

At the end of the 4-day application period, erythema was assessed in all subjects by a visual scoring system using a 10-point scale (0 = absent; 1–3 = mild; 4–6 = moderate; 7–9 = severe) and by the a^* value of the L^*a^* b^* scale system as measured by colorimeter (Minolta chromameter CR-200, Minolta, Osaka, Japan). In 10 subjects, 6 mm punch biopsies were taken for measurement of CRABP-II mRNA levels by the semiquantitative RT-PCR technique, using mRNA for the enzyme GAPDH as an internal reference. In another 11 subjects, epidermal thickness from the base of the stratum corneum to the basement membrane of the inter rete ridges was assessed using image analysis (Quantimet 600S) of haematoxylin and eosin stained sections from 4-mm punch biopsies.

Statistical methodology

Statistical analysis was conducted using analysis of variance for continuous variables and two-sided rank sign test for erythema scores. CRABP-II levels were logarithmically transformed before analysis. Each active product was compared to its corresponding vehicle (this was thus not possible for the tretinoin 0·1% cream formulation), and the two adapalene formulations were each compared to tretinoin 0·05% cream and tretinoin 0·1% cream. Statistical significance was defined as $P < 0{\cdot}01$ to compensate for multiple comparisons.

Results

CRABP-II

As shown in Fig. 1, adapalene 0·1% gel and adapalene 0·1% cream each produced approximately 2-fold increases in CRABP-II mRNA levels compared to their vehicles ($P \leqslant 0{\cdot}001$). The two tretinoin formulations resulted in 3-to 4-fold increases in CRABP-II ($P < 0{\cdot}001$ for comparison of 0·05% concentration vs. vehicle).

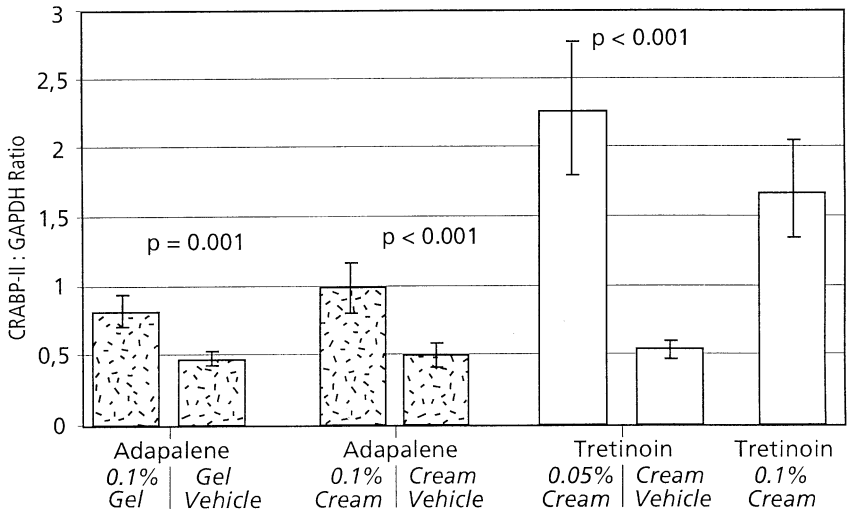

Figure 1. Levels of CRABP-II mRNA in skin treated for 4 days under occlusion with adapalene, tretinoin or vehicle. Both formulations of adapalene produce significantly more CRABP-II gene expression than vehicle. Values are means ± SEM, $n = 10$.

Epidermal Thickness

As shown in Fig. 2, only the two tretinoin formulations resulted in an increase in epidermal thickness, which amounted to 30% for the tretinoin 0·05% cream ($P < 0·01$) and 74% for the tretinoin 0·1% cream.

Erythema

As shown in Fig. 3, only the tretinoin 0·1% cream formulation resulted in clinically significant erythema (mean score of 3 compared to less than 1 for each of the other products). According to the colorimetry a^* readings (Fig. 4), which represent an objective measure of skin redness, the erythema produced by tretinoin 0·1% cream was significantly greater than that by either adapalene formulation ($P \leqslant 0·001$). See also Figs 5 and 6 for an illustration of the appearance of typical subjects after application of the different products.

Discussion

The results of this study are consistent with those of previously published data[7] which showed that adapalene was able to induce expression of mRNA for CRABP-II (a marker of nuclear retinoic acid receptor agonist activity) while not inducing erythema or epidermal hyperplasia (which appear to be the result of non-specific irritant activity of all *trans* retinoic acid[6]). In turn, the results of this short-term model correlate well with those of clinical trials in acne patients, where adapalene was equally efficacious as tretinoin, but significantly better tolerated.[3,4]

Interestingly, the gel and cream formulations of adapalene gave the same degree of CRABP-II expression, and a similar absence of signs of irritation. On the other hand, the tretinoin 0·05% cream formulation appeared to induce much less erythema and epidermal hyperplasia than tretinoin 0·1% cream, while main-

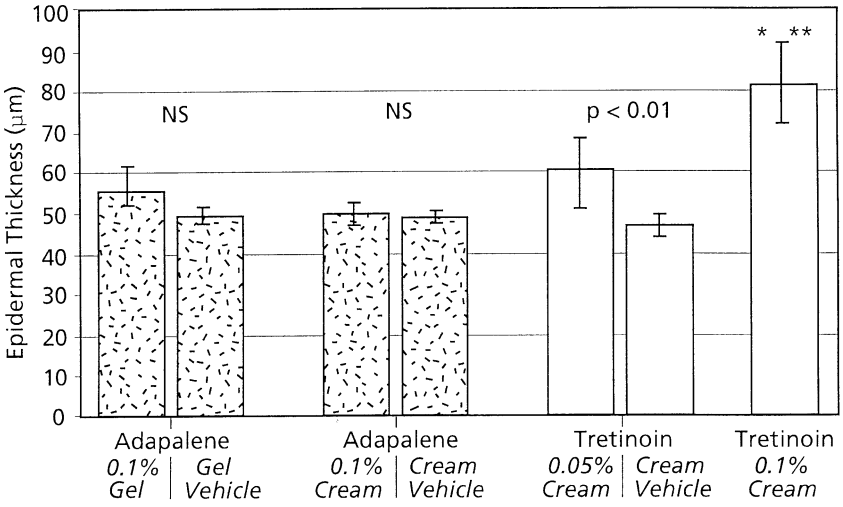

Figure 2. Epidermal thickness of skin treated for 4 days under occlusion with adapalene, tretinoin or vehicle. There is no significant difference in epidermal thickness between either formulation of adapalene and their vehicles, but tretinoin produces significant epidermal thickening. Epidermal thickness is measured from the base of the stratum corneum to the basement membrane of the inter rete ridges. Values are means ± SEM, $n = 11$. $^*P = 0·011$ vs. adapalene gel, $^{**}P = 0·001$ vs. adapalene cream.

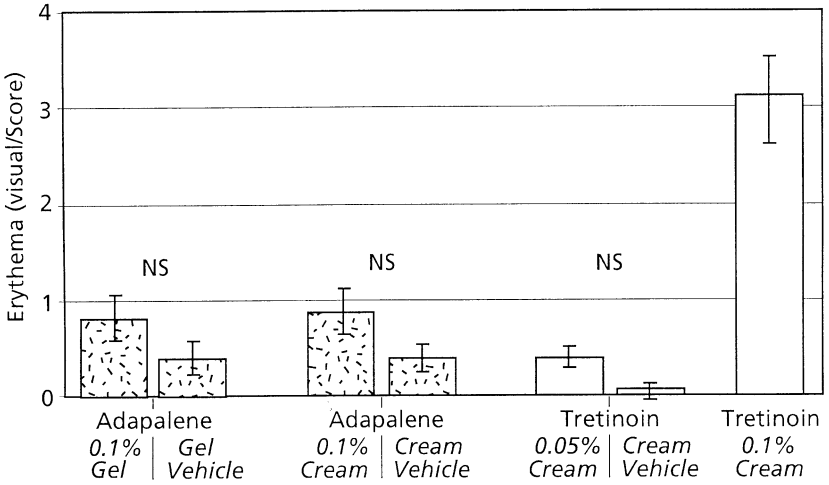

Figure 3. Erythema (visual score) of skin treated for 4 days under occlusion with adapalene, tretinoin or vehicle. Tretinoin 0·1%, but neither tretinoin 0·05% or adapalene, produced significant erythema. Erythema was measured on a 0–9 ordinal scale. Values are means ± SEM, $n = 30$

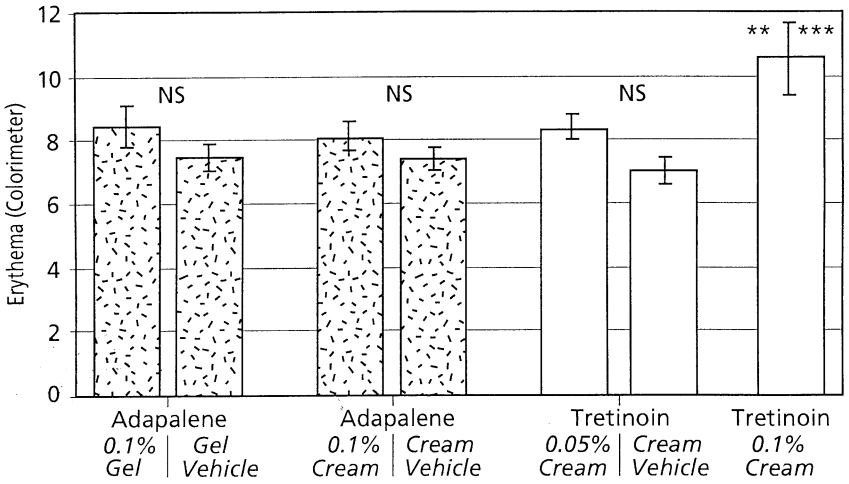

Figure 4. Erythema (colorimetry measurement) of skin treated for 4 days under occlusion with adapalene, tretinoin or vehicle. Tretinoin 0·1%, but neither tretinoin 0·05% or adapalene, produced significant erythema as measured by colorimeter. Values are means ± SEM, $n = 30$. $^{**}P = 0{\cdot}001$ vs. adapalene gel, $^{***}P < 0{\cdot}001$ vs. adapalene cream.

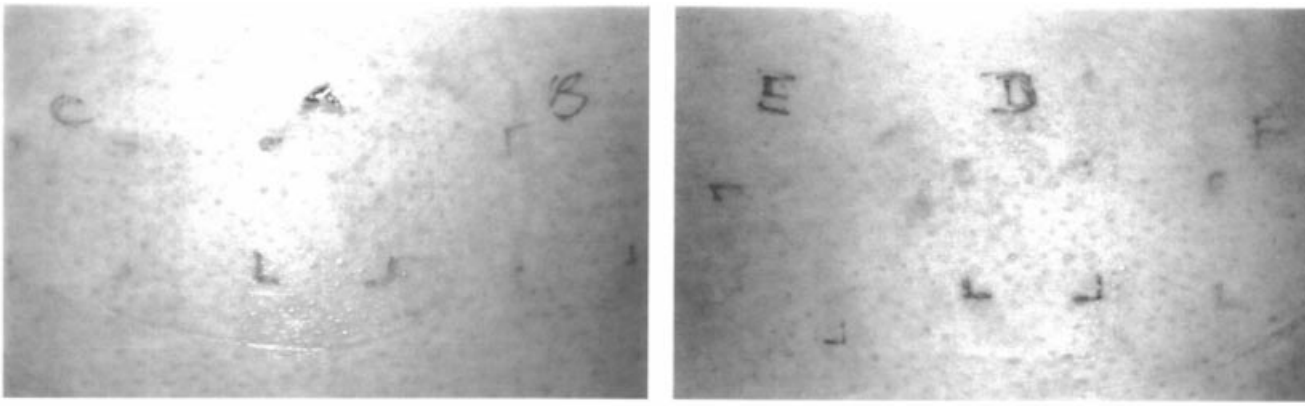

Figure 5. Subject 4. From left to right: C = tretinoin 0·05% cream, A = adapalene 0·1% gel, B = adapalene 0·1% cream, E = adapalene cream vehicle, D = adapalene gel vehicle, *F* = tretinoin cream vehicle.

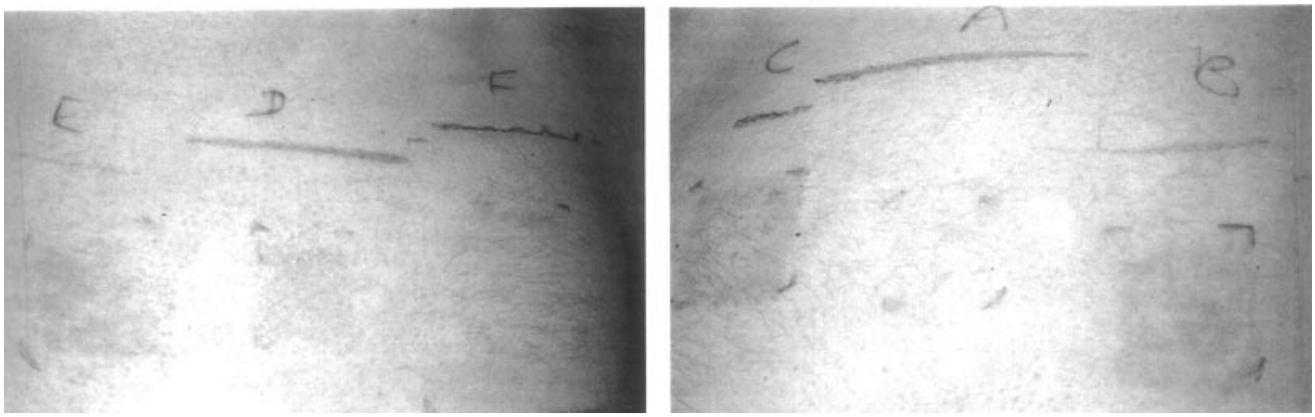

Figure 6. Subject 19. From left to right: E = adapalene cream vehicle, D = adapalene gel vehicle, **F** = tretinoin cream vehicle, C = tretinoin 0·1% cream, A = adapalene 0·1% gel, B = adapalene 0·1% cream.

taining at least as much increase in CRABP-II expression. The latter finding may be explained either by CRABP-II expression being a more sensitive marker of retinoid activity, or alternatively by the influence of the vehicle formulation on the skin tolerance profile. Unfortunately, the tretinoin 0·1% vehicle was not tested so direct comparisons cannot be made. Nevertheless, the findings further support the hypothesis that retinoid agonist activity is not necessarily proportional to the degree of skin irritation, and that the two

components can be readily distinguished using this rapid 4-day occlusion assay.

References

1 Shroot B, Michel S. Pharmacology and chemistry of adapalene. *J Am Acad Dermatol* 1997; **36:** S96–103.

2 Verschoore M, Poncet M, Czernielewski J *et al.* Adapalene 0·1% gel has low skin irritation potential. *J Am Acad Dermatol* 1997; **36:** S104–9.

3 Clucas A, Verschoore M, Sorba V, Poncet M, Baker M, Czernielewski J. Adapalene 0·1% gel is better tolerated than tretinoin in acne patients. *J Am Acad Dermatol* 1997; **36:** S104–9.

4 Shalita A, Weiss JS, Chalker DK *et al.* A comparison of the efficacy and safety of adapalene gel 0·1% and tretinoin gel 0·025% in the treatment of acne vulgaris: a multicenter trial. *J Am Acad Dermatol* 1996; **34:** 482–5.

5 Elder JT, Cromie MA, Griffiths CEM *et al.* Stimulus-selective induction of CRABP II mRNA. A marker for retinoic acid action in human skin. *J Invest Dermatol* 1993; **100:** 356–9.

6 Griffiths CEM, Finkel LJ, Tranfalgia MG *et al.* An *in vivo* experimental model for effects of topical retinoic acid in human skin. *Br J Dermatol* 1993; **129:** 389–94.

7 Griffiths CEM, Elder JT, Bernard BA *et al.* Comparison of CD271 (adapalene) and all-*trans*-retinoic acid in human skin: dissociation of epidermal effects and CRABP II mRNA expression. *J Invest Dermatol* 1993; **101:** 325–8.

8 Loesche C, Tuley M, Dupre S. Studies of Cutaneous tolerance with adapalene cream, 0·1%. Poster N° 13 presented at 58th Annual Meeting of the American Acadamy of Dermatology, Orlando, February 26th–March 4th 1998.