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ORIGINAL ARTICLE

Adapalene biochemistry and the evolution of a new topical retinoid for treatment of acne

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ABSTRACT

The emergence of oral and topical retinoids was a major advance in the clinical management of acne vulgaris. However, the benefits of these agents were somewhat limited by the degree of side effects caused by these drugs. Over the last 15 years, researchers have sought compounds that can provide the manifold therapeutic benefits obtained with tretinoin and isotretinoin while minimizing the potential for irritation and other unwanted effects. Adapalene, a naphthoic-acid derivative, is one result of this search, and it serves as an example of rational drug development: the formulation of a novel substance with specific pharmacological properties and clinical objectives in mind. These goals included enhancing stability, enhancing anti-inflammatory effects, maintaining effectiveness and minimizing cutaneous irritation. This paper reviews the history of the development of adapalene, its unique physical and biochemical properties, and the pharmacological studies that demonstrate a wide range of retinoid-receptor, genetic and anti-inflammatory effects, all of which contribute to the therapeutic efficacy and improved tolerability of adapalene observed in the clinical use of this agent for the treatment of acne.

Key words: adapalene, biochemistry, pharmacology

Introduction

The introduction of retinoids for the treatment of acne vulgaris in the 1970s was nothing short of revolutionary. For the first time, dermatologists had effective comedolytic medication that could attenuate multiple aspects of acne pathogenesis, resulting in superior clinical and cosmetic outcomes. The pathogenesis of acne is multifactorial, with four main mechanisms: abnormal proliferation and differentiation of follicular keratinocytes; increased production of sebum; colonization of the pilosebaceous unit by *Propionibacterium acnes*; and initiation of inflammatory reactions by bacterial antigens and pro-inflammatory factors. With the exception of a direct antibacterial effect, retinoids can positively influence all of these mechanisms.

The benefits of topical tretinoin and all first- and second-generation retinoids has been somewhat limited by the relatively high prevalence of irritation resulting from these medications. While usually mild, retinoid-associated irritation adversely affects patient compliance, and is the primary reason patients discontinue treatment.

Almost as soon as topical tretinoin became available for widespread use, clinical researchers and pharmaceutical company research-and-development scientists began a search for compounds that could provide similar multifocal effects and clinical efficacy, while minimizing the potential for irritation and other unwanted side effects.

Galderma's efforts in this direction ultimately resulted in the development of adapalene, a naphthoic-acid derivative with retinoid activity. The drug was launched as a topical 0.1% gel formulation in 1995, after 10 years of basic research, preclinical testing and clinical trials. The development process was driven by the need for an agent that could offer all of the therapeutic benefits of tretinoin while minimizing the burden of retinoid-associated irritation. The pharmacological and clinical objectives were clear in the early 1980s, and in many ways adapalene is an example of rational drug development: the creation of a therapeutic agent in accordance with very specific pharmacological and clinical objectives.

Chemical stability of adapalene vs. tretinoin

The first step in the evolution of adapalene was an attempt to understand why tretinoin, then the leading topical retinoid, is so irritating. Hans Schaeffer, PhD, then director of R & D at Galderma, forwarded a hypothesis that the irritant potential of

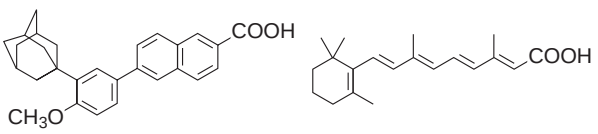
tretinoin was due to its molecular instability owing to a series of fragile double bonds. Various products of tretinoin degradation have been shown to produce erythema, stinging, burning sensations, skin dryness – essentially all of the adverse effects seen in clinical practice.[1]

We showed, in the laboratory, that tretinoin can be degraded by many different factors. When exposed to normal daylight, there is a 60–80% degradation of a baseline concentration of tretinoin within 24 h. An oxidizing agent like benzoyl peroxide, used commonly by acne patients, induces 80% degradation of tretinoin within as little as 4 h; nearly all tretinoin is lost within 24 h in the presence of benzoyl peroxide.[2]

The search for a more stable molecule capable of producing therapeutic effects similar to tretinoin led to the development of adapalene, a completely novel chemical entity derived from naphthoic acid (fig. 1) (Table 1). Essentially, the chain of unstable double bonds characteristic of tretinoin was replaced by naphthoic acid aromatic rings, which gave the adapalene molecule far greater stability in conditions of light and oxygen exposure. When exposed to light or benzoyl peroxide, the adapalene molecule maintains its integrity with essentially no degradation, even after 72 h of exposure.[2] The phenoxy adamantyl portion of the molecule gives it low skin flux, thus enhancing the safety profile, and good lipophilicity that is essential for good penetration of the affected follicles in acne.

Pharmacology of adapalene

Once a more stable and potentially less irritating molecule had been synthesized, the next challenge was to determine to what extent the new compound could provide the clinically important changes that lead to the clinical benefits seen with topical tretinoin. To determine this, we evaluated the new molecule's ability to bind to retinoid receptors, and to influence clinically relevant biological processes.



Adapalene Tretinoin

fig. 1 Novel chemical structure of adapalene.

Table 1 Novel chemical structure of adapalene

Group	Property	Consequences
Naphthoic acid	Aromatic	Stable to oxygen/light
Phenoxy adamantyl	High melting	Low flux through skin
Phenoxy adamantyl	Low solubility	High levels in skin

Retinoid-receptor binding profile

Adapalene selectively binds to retinoic acid receptors, and can modulate gene transactivation and gene expression in human keratinocytes. Hence, it is able to regulate proliferation and differentiation of these cells, which are key players in the evolution of acne lesions.

We compared the binding affinity of tretinoin and adapalene for the three different subtypes of retinoic acid receptors (RAR): RAR-alpha, RAR-beta, and RAR-gamma. Tretinoin showed more or less equal affinity for all three receptor subtypes, whereas adapalene showed a 10-fold lower affinity for RAR-alpha, but binds strongly to RAR-beta and -gamma. This is an important property because RAR-gamma is the predominant receptor found in epidermal and epithelial cells.[3–6]

Adapalene binds tightly to specific amino acids in the binding pocket of the receptors, and this complex of receptor–agent interacts with a different type of receptor molecule, retinoic X receptor (RXR). The complex of RAR with adapalene and RXR can then bind to DNA at specific sites, leading to modulation of the transcription machinery. This translates into changes in protein synthesis that can affect cell proliferation and differentiation. In essence, all retinoids are able to alter the phenotype of cells.

Anti-proliferative effects and modulation of cell differentiation

Tretinoin has well-established *in vitro* effects in reducing keratinocyte proliferation. In the early 1980s a series of studies began to compare the effect of adapalene against that of tretinoin on the proliferation of the experimental HeLa cell line, as well as on the differentiation of F9 embryonic carcinoma cell lines and normal human keratinocytes. Adapalene and tretinoin both showed strong inhibitory activity on HeLa cell proliferation, although tretinoin was somewhat stronger by a factor of 2.7.[7] However, adapalene showed a fivefold greater effect in preventing F9 cell end-stage differentiation.[8] The inhibitory effect on human keratinocytes was assessed by measuring keratinocyte transglutaminase expression, a marker for keratinocyte differentiation. Adapalene showed greater (10-fold) inhibition of keratinocyte transglutaminase expression,[9] suggesting that it is a strong inhibitor of keratinocyte differentiation (Table 2). We believe keratinocytes

Table 2 *In vitro* studies: anti-proliferative and differentiation activity

Compound	Prolif. HeLa IC_{50}(nM)	Diff. F9 AC_{50}(nM)	NHK TG 1 Expression IC_{50}(nM)
Tretinoin	6	200	24
Adapalene	16	40	2.5
Ratio	2.7 : 1	1 : 5	1 : 10

may be one of the key target cells for the therapeutic action of adapalene.

To test this, we measured the effect of adapalene on a reconstructed epidermis model, using keratin-10 synthesis as a marker for keratinocyte differentiation. Keratin-10 expression was quantified using a fluorescent antibody to identify this molecule. Adapalene had a marked effect in reducing keratin-10 synthesis (fig. 2); inhibition was nearly total. Addition of CD 2665, a molecule that is an RAR-beta, RAR-gamma antagonist, completely inhibited this effect of adapalene. Because RAR-beta is not expressed in the epidermis, the offset of the adapalene effect by CD 2665 confirms the preferential binding of adapalene for the RAR-gamma retinoid receptor in the epidermis.[9,10]

Comedolytic activity

Keratinocyte hyperproliferation is one of the major pathogenic processes driving the development of microcomedones, which are essential to the development of acne vulgaris. The modulation of differentiation observed *in vitro* strongly suggested that, like tretinoin, adapalene would be comedolytic.

One of the major steps in the evaluation of adapalene was the development of an animal model, the rhino mouse, for studying comedogenesis and the comedolytic activity of retinoids. The rhino mouse has a genetically induced cutaneous structural abnormality that results in the complete loss of fur at approximately 4 weeks of life, and the development of a wrinkled skin, rich in sebaceous glands. Histologically, the skin of this mouse is characterized by high-density, keratin rich utriculi, which show a marked resemblance to human acne comedones (fig. 3). When treated with adapalene, both the mouse utriculi and human comedones show the same sort of changes: expression of the sebum-rich keratin plug to the surface of the skin, reduced keratinocyte proliferation, and a normalization of the follicle.[11,12]

We quantified these follicular changes by the comedone profile, that is a ratio between the smallest and largest diameters of each urticuli, expressed as a percent.[11] In general, comedones have a very narrow diameter at the top of the follicle, and owing to the distention caused by the keratin plug, a very large diameter at the infundibulum. Thus, untreated active comedones tend to have lower comedone profiles, whereas a normalized follicle has a higher ratio.

We measured both comedone counts and comedone profiles in the rhino mouse treated with adapalene cream 0.1%, adapalene gel 0.1%, and tretinoin gel 0.025%. At both measurements, the three treatments were nearly identical. All yielded comedone-count reductions between 50 and 60% compared with vehicle, and likewise gave the expected increases in comedone profiles, from just under 100% for the vehicle to well over 200% for the three active treatments (fig. 4).[11]

Anti-inflammatory activity

Dermatological researchers and clinicians alike have begun to realize the importance of immunological and inflammatory processes in the evolution of the microcomedo and the exacerbation of acne. To improve the benefit of acne treatment it is necessary to address this aspect of the pathogenesis.

Much of the clinical efficacy seen with topical tretinoin reflects its ability to modulate various components of the immune system and its inflammatory responses to *P. acnes*-induced antigens and inflammatory mediators. Any agent that purports to be an improvement on tretinoin must be able to demonstrate similar or better anti-inflammatory activity. Pharmacological studies indicated that adapalene affects a number of important inflammatory processes.

Because polymorphonuclear (PMN) leucocytes are important cells in the inflammatory response to *P. acnes*, we tested adapalene and other compounds in cultures of PMN leucocytes isolated from human blood. The anti-inflammatory properties of adapalene were assessed using a number of different experimental protocols. Initially, we compared adapalene with tretinoin, and with two well-established anti-inflammatory agents: indomethacin, a non-steroidal anti-inflammatory, and betamethasone-17-valerate, a corticosteroid. We were able to demonstrate that adapalene is a strong inhibitor of PMN lipoxygenase activity, whereas tretinoin showed far less activity.[13]

Adapalene also proved to be a good inhibitor of arachidonic acid-induced ear oedema in a mouse model. The arachidonic-acid cascade is an essential pathway for the production of leukotrienes, so the inhibitory action of adapalene suggests that it can attenuate leukotriene-dependent inflammatory mechanisms. Indomethacin and, to a lesser extent, betamethasone were also strong inhibitors of arachidonic-acid induced oedema. Tretinoin had very little inhibitory activity against the arachidonic-acid cascade.[14]

To explore a different aspect of the inflammatory response, we tested the ability of the two retinoids and the two anti-inflammatory agents to inhibit croton-oil induced skin inflammation in rats. The form of inflammation generated by croton oil can be inhibited by corticosteroids, but not by non-steroidal anti-inflammatory agents. The effect of retinoids had not previously been determined.[13]

As expected, the betamethasone-17-valerate had a strong inhibitory effect, whereas the indomethacin was almost completely inactive. Tretinoin showed very little inhibitory activity. Surprisingly, adapalene had a moderate inhibitory effect, suggesting that it influences different aspects of the inflammatory response to tretinoin.[13]

We also tested the four agents as inhibitors of carageenan-induced inflammation in rats. This model is well recognized as a way to preferentially measure the activity of non-steroidal anti-inflammatory agents. Not surprisingly, indomethacin

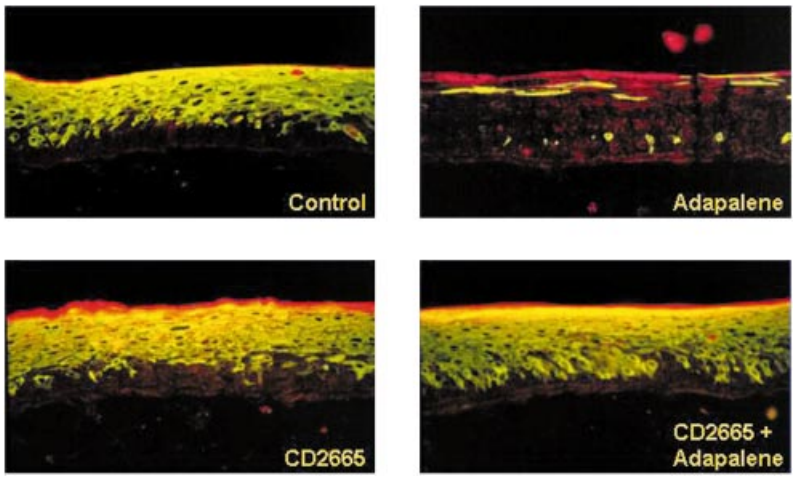


fig. 2 Effect of CD2665 on the effect of adapalene on reconstructed skin: K-10 expression.

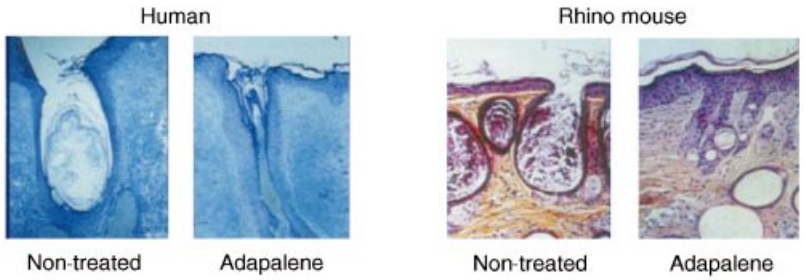


fig. 3 Rhino mouse model of comedogenesis.

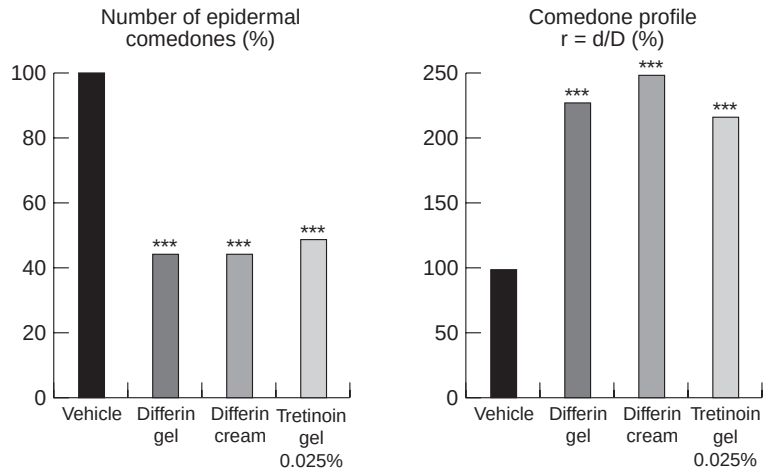


fig. 4 Comedolytic properties in the rhino mouse.[11] ***$P < 0.001$.

showed a strong inhibitory effect, whereas betamethasone-17-valerate was only moderately active. Adapalene was equivalent to betamethasone and had a greater inhibitory effect than tretinoin. Carageenan-induced inflammation results from leucocyte migration and prostaglandin E2 synthesis. The findings indicate that adapalene has significant effects in inhibiting these processes.[13]

One way that retinoids modulate keratinocyte gene expression is by inhibition of the AP-1 pathway. AP-1 is a transcription factor

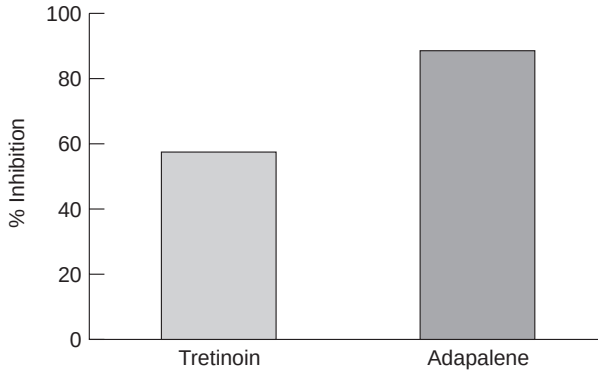


fig. 5 Effects of tretinoin and adapalene on AP-1 activity.

that mediates an aspect of the inflammatory response triggered by UV-light irradiation. AP-1 controls expression of several genes involved in inflammation, including the genes coding for vascular endothelial growth factor and matrix metalloproteins. Tretinoin and adapalene both show inhibitory activity against AP-1 (fig. 5). *In vivo* studies with mice and guinea pigs suggest that adapalene can reduce UV-induced erythema.[15]

Irritation and tolerability

The primary objective in the development of adapalene was to create a topical agent with retinoid therapeutic effects that is considerably less irritating than topical tretinoin. To date, 20 studies involving more than 400 volunteers have tested the safety and tolerability of adapalene according to standard Phase I protocols. Cumulative results of these studies clearly show that adapalene is a very well tolerated compound with markedly lower irritation potential as compared with tretinoin.

Irritation potential

Repeated 21-day patch testing under occlusion showed that adapalene has a very low potential for irritation. Using a 0–4 cumulative irritancy index, the aggregate data for 22 volunteers who underwent patch testing showed that adapalene gel 0.03% and 0.1% each had index scores of 0.19, comparable to a gel vehicle, with an index of 0.07, and the untreated skin patch, with an index of 0.06. In essence, there was little difference between adapalene-treated skin and the ordinary baseline irritation potential in normal untreated skin. In contrast, tretinoin gel 0.025% had a cumulative irritancy index score of 3.42, a marked difference from both vehicle and untreated skin.[16]

In a later 21-day cumulative irritation index study, we compared adapalene 0.1% gel with three different concentrations of tretinoin cream (0.025%, 0.05% and 0.1%) and two different concentrations of tretinoin gel (0.1% and 0.025%), as well as the new tretinoin 0.1% gel microsphere formulation. Petrolatum was used as a control. There were no differences in mean daily irritation scores over the 21 days for adapalene and petrolatum, whereas all forms of tretinoin began to show increased irritation scores by the fourth day of treatment (fig. 6). Tretinoin 0.1% cream induced the highest degree of irritation, with the score increasing sharply from 0 to 1.5 by day 4, and peaking at 2.25 by day 15. The other tretinoin preparations, including the microsphere gel, showed similar patterns of rapidly increasing irritation scores, although none were as irritating as the tretinoin 0.1% cream.[17]

In an effort to determine how adapalene might fit into topical combination regimens, we carried out a 21-day cumulative irritation study in 25 patients comparing monotherapy with adapalene gel 0.1% against monotherapy with benzoyl peroxide 10%, erythromycin 4%, and clindamycin phosphate 1%. We also compared adapalene monotherapy against adapalene in combination with each of these other three antimicrobials.[18] The two most irritating treatments were benzoyl peroxide alone and benzoyl peroxide in combination with adapalene. However, the cumulative irritation scores were so low as to be almost meaningless, thus demonstrating the compatibility and tolerability of adapalene with other anti-acne agents.

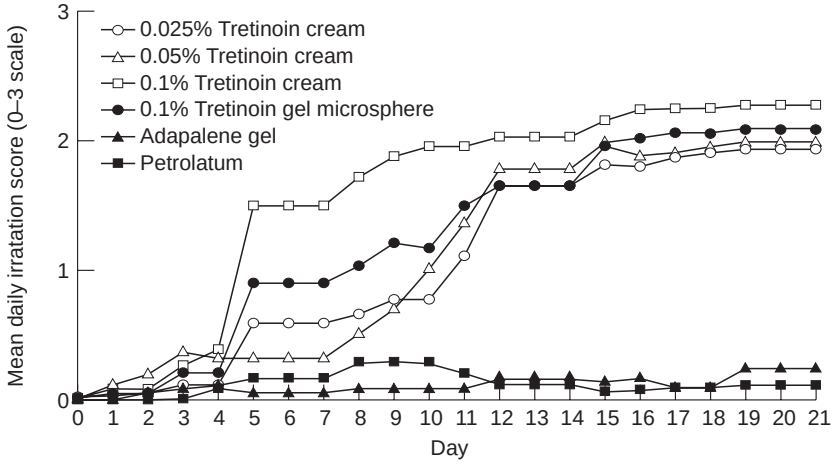


fig. 6 Adapalene vs. tretinoin: 21-day cumulative irritation.

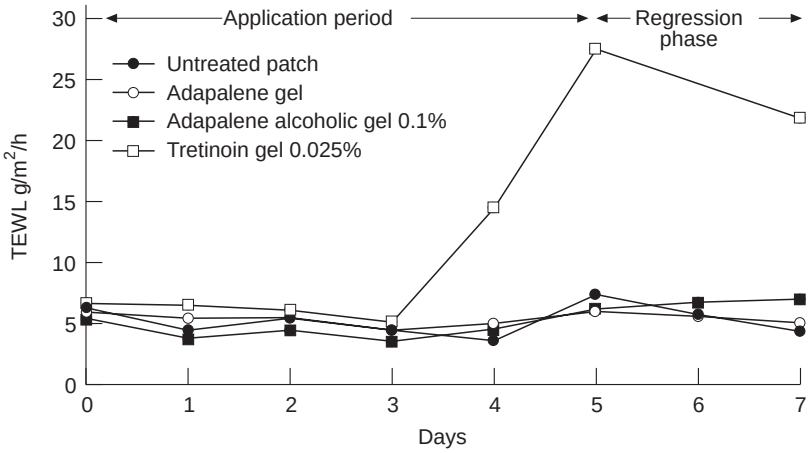


fig. 7 Transepidermal water loss measurement on forearms (n = 20).

Measurement of transepidermal water loss (TEWL) is another means of assessing irritation potential because it reflects the integrity of the stratum corneum. Whenever the stratum corneum is compromised, there is an increase in TEWL. We measured TEWL in the forearm skin of 20 volunteers treated with tretinoin gel 0.025%, adapalene 0.1% in the standard water-based gel, and adapalene 0.1% in a specially prepared alcohol gel designed to mimic the alcohol content of the tretinoin,[16] thus controlling for the irritant effect of an alcohol-based product.

After 7 days of exposure, both formulations of adapalene showed no change in TEWL from baseline. The measurements were essentially equivalent to untreated skin. In contrast, for the tretinoin-treated patch, the TEWL increased from just over 5 g/m²/h to 27 g/m²/h by treatment day 5. For the first 3 days of treatment, there was no noticeable increase in TEWL in the tretinoin-treated skin; it began to increase between days 3 and 4, peaking on day 5 (fig. 7). The fact that an alcohol-based preparation of adapalene did not induce any measurable change in TEWL counters the argument that tretinoin-associated irritation can be attributed to its alcohol formulation.[16]

Systemic absorption

One of the chief virtues of a topical approach to treating skin disease is the minimization of systemic side-effects. This is contingent on minimal systemic absorption of the topical agent. We measured systemic absorption of adapalene gel 0.1% in six patients with acne vulgaris. Patients were treated daily with 2 g of the gel for a total of 14 days. The drug was applied to the face, chest and shoulders, covering a total of approximately 1000 cm². Only one of a total of 108 plasma samples had any measurable level of adapalene (0.38 ng/mL). Two samples had trace levels in the 0.15–0.25 ng/mL range. (*Data on file.*)

By and large, any absorbed adapalene is excreted in the faeces, peaking between day 10 and 11 at a level of 30 ng/g of applied adapalene. This is still very low, suggesting minimal systemic absorption of topically applied adapalene.

Clinical evaluation of adapalene

All preclinical and early clinical work strongly suggested that adapalene could meet the stated objective: creation of a topical treatment with retinoid-like benefits but without the retinoid-associated irritation and adverse effects profile. The next stage in development of the product was an exhaustive programme of efficacy and safety clinical trials that began in 1986 with the initial 8-week treatment trial involving 29 patients with acne vulgaris. Fifteen were randomized to active adapalene therapy, while 14 were treated with vehicle only. We observed a reduction in total number of comedones of almost 70% in the adapalene group, compared to 30% in the vehicle-treated patients. Inflammatory lesions were slower to respond, but we still saw a 35% reduction in mean inflammatory lesion count by week 8 in the adapalene group, as compared to a 10% reduction in the vehicle-treated patients.[19]

A second dose-ranging trial, conducted in France and Poland, compared adapalene 0.03% and adapalene 0.1% vs. tretinoin 0.025% gel in terms of non-inflammatory and inflammatory lesion reduction. A total of 89 patients were enrolled and randomized to each treatment group. All three treatments produced steady reductions in lesion counts over the 12-week course of treatment.[20] By the end of the study, each of the groups had mean non-inflammatory lesion reductions in the range of 65% to 80%.

Inflammatory lesion responses were somewhat lower, although all three treatments produced substantial reductions from baseline, in the range of 65–70% for tretinoin 0.025% and adapalene 0.1%, and 40% for adapalene 0.03%. The investigators concluded that both adapalene gels are safe and effective, with a dose-related response. Adapalene 0.1% and tretinoin 0.025% had equivalent efficacy, but adapalene 0.1% was found superior to tretinoin in skin tolerance. Adapalene 0.1% became the standard concentration for subsequent adapalene formulations.[20]

These initial clinical trials paved the way for a series of larger-scale multi-centre trials in the US and Europe involving a cumulative total of 3878 patients, comparing adapalene 0.1%

gel or solution vs. tretinoin 0.025% gel.[21–25] Results of these studies consistently showed adapalene to be equivalent to or slightly more effective than tretinoin in reducing inflammatory, non-inflammatory and total lesion counts. All studies found adapalene preparations significantly less irritating than tretinoin.

Discussion

The ideal topical treatment for acne vulgaris would exert therapeutic effects on all four key components of the pathogenic process: abnormal keratinocyte proliferation and differentiation; increased sebum production; colonization of the pilosebaceous unit with *P. acnes*; and inflammatory reactions to *P. acnes*-derived antigens and mediators. It would achieve these therapeutic goals without skin irritation or any other adverse effects.

From the earliest days of the retinoid era, clinicians and researchers recognized that the value of these agents would be limited to some degree by their propensity to induce skin irritation, which remains the primary reason patients discontinue retinoid therapy. Since the early 1980s, considerable research and development effort has been spent seeking novel compounds that give the therapeutic benefits of retinoids but without the concomitant irritation.

Pharmacological studies indicate that, like tretinoin, adapalene binds to retinoic-acid receptors and influences several components of the inflammatory response. However, it has a unique binding profile, showing particular affinity for the epidermal RAR-gamma receptor. Adapalene has been shown to inhibit polymorphonuclear leucocyte and arachidonic acid-mediated immune responses. It also shows powerful inhibitory effects on keratinocyte proliferation and differentiation, which has been borne out clinically as strong comedolytic activity.

Clinical trials involving nearly 4000 acne patients in the US and Europe have consistently shown adapalene 0.1% solution or gel to be as effective or superior to tretinoin 0.025% in reducing inflammatory, non-inflammatory and total lesion counts. All studies have shown adapalene to be less irritating than tretinoin, as would be expected given its pharmacological properties.

We believe adapalene offers significant advantages over tretinoin, and that it stands as an example of what can be achieved through a programme of rational drug development guided by clear, clinically relevant objectives.

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