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Acne Treatment Methodologies

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INTRODUCTION

Acne is an exceedingly common condition affecting millions of adolescents and young adults. Not surprisingly, the psychological and economic impact of acne is reflected in these vast numbers. The prevalence in teenage girls ranges from 16–80%, while teenage boys are even more likely to be affected with prevalence ranging from 29–90% (1–4). These large variations in prevalence are due to differences in acne grading scales used in the various studies. Adult acne, although less common than adolescent acne, continues to be a significant problem for 3–6% of adult men, and 5–12% of adult women well into their thirties and forties (5,6). With so many persons affected, the economic impact of acne is immense. In 1999 there were approximately 35 million Americans with acne generating 7.9 million physician visits. That same year approximately 1.2 billion dollars was spent on prescription acne medications (7).

In addition to the economic impact, acne also has a significant psychological impact in both adolescents and adults.Thirty to fifty percent of adolescents experience psychiatric disturbances due to acne (8). Studies have shown that acne causes similar levels of social, psychological, and emotional impairment as asthma and epilepsy (9). Studies have also shown that unemployment is higher among adults with acne than among adults without acne (10).

In order to implement effective treatment strategies for patients with acne, a solid understanding of the physiology of the pilosebaceous unit and the pathological events that lead to acne are essential. The pathogenesis of acne is very complex, but four basic steps have been identified. These key elements (Fig. 1) are: (i) follicular epidermal hyperproliferation, (ii) excess sebum production, (iii) inflammation, and (iv) the presence and activity of *Propionibacterium acnes* (P. acnes).

Follicular Epidermal Hyperproliferation

Follicular epidermal hyperproliferation results in the formation of the primary lesion of acne, the microcomedo. The epithelium of the upper hair follicle, the infundibulum, becomes hyperkeratotic with increased cohesion of the keratinocytes. The excess cells and their tackiness result in a plug in the follicular ostium. This plug then causes downstream concretions of keratin, sebum, and bacteria to accumulate in the follicle. These packed concretions cause dilation of the upper hair follicle producing a microcomedo. The stimulus for keratinocyte hyperproliferation and increased adhesion is unknown. However,

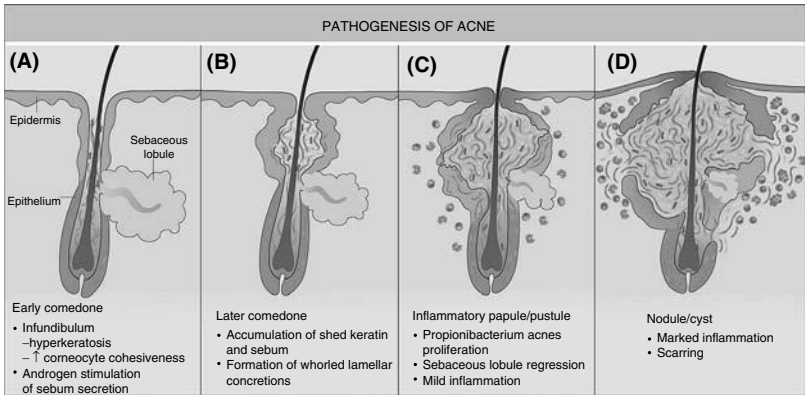

Figure 1 Acto Pathogenesis. The four key steps in acne pathogenesis: (**A**) follicular epidermal hyperproliferation, (**B**) excess sebum production, (**C**) inflammation, and (**D**) the presence and activity of *Propionibacterium acnes*. *Source*: Adapted from Ref. 11.

several proposed factors in keratinocyte hyperproliferation include: androgen stimulation, decreased linoleic acid, and increased interleukin-1 alpha (IL-1 alpha) activity.

Androgenic hormones may act on the follicular keratinocytes stimulating hyperproliferation. Dihydrotestosterone (DHT) is a potent androgen that may play a role in acne. Figure 2 demonstrates the physiologic pathway for dehydroepiandrosterone sulfate (DHEA-S) conversion to the androgen DHT. 17-beta HSD and 5-alpha reductase, are enzymes responsible for converting DHEA-S to DHT. When compared to epidermal keratinocytes, follicular keratinocytes have increased 17-beta HSD, and 5-alpha reductase thus enhancing DHT production (12,13). DHT may stimulate follicular keratinocyte proliferation. Also supporting the role of androgens in acne pathogenesis is the evidence that the individuals with complete androgen insensitivity do not get acne (14).

Follicular keratinocyte proliferation may also be regulated by linoleic acid. Linoleic acid is an essential fatty acid in the skin that is decreased in subjects with acne. The quantity of linoleic acid normalizes after successful treatment with isotretinoin. Subnormal levels of linoleic acid may induce follicular keratinocyte hyperproliferation, and produce pro-inflammatory cytokines. It has also been suggested that regular quantities of linoleic acid are actually produced but are simply diluted by increased sebum production (15).

In addition to androgens and linoleic acid, IL-1 may also contribute to keratinocyte hyperproliferation. Human follicular keratinocytes demonstrate hyperproliferation and microcodemo formation when IL-1 is added. IL-1 receptor antagonists inhibit

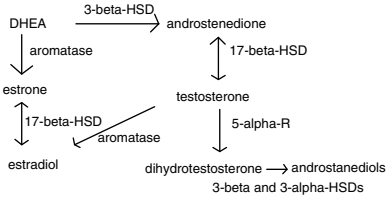

Figure 2 Steroid metabolic pathway. DHEA is a weak androgen that is converted to the more potent testosterone by 3-beta-HSD and 17-beta-HSD. Five-alpha-reductase then converts testosterone to dihydrotestosterone, the predominant hormonal effector on the sebaceous gland. Both DHEA and testosterone can be metabolized to estrogens by the enzyme aromatase. The sebaceous gland expresses each of these enzymes. *Source*: Adapted from Ref. 11.

microcodemo formation providing additional support for the cytokine's role in acne pathogenesis (16,17).

Excess Sebum Production

The second key feature in the pathogenesis of acne is excess sebum production from the sebaceous gland. Patients with acne produce more sebum than those without acne although the quality of sebum is the same between the two groups (18). One of the components of sebum, triglycerides, may play a role in acne pathogenesis. Triglycerides are broken down into free fatty acids by *P. acnes*, normal flora of the pilosebaceous unit. These free fatty acids promote further bacterial clumping and colonization of *P. acnes*, incite inflammation, and may be comedogenic (19).

Androgenic hormones also influence sebum production. Similar to their action on the follicular infundibular keratinocytes, androgen hormones bind to and influence sebocyte activity (20). Those with acne have higher average serum androgen levels (although still within normal range) than unaffected controls (21,22). 5-alpha reductase, the enzyme responsible for converting testosterone to the potent DHT, has greatest activity in areas of skin prone to acne, the face, chest, and back (23).

The role of estrogen on sebum production is not well defined. The dose of estrogen required to decrease sebum production is greater than the dose required to inhibit ovulation (24). The mechanisms by which estrogens may work include: (i) directly opposing the effects of androgens within the sebaceous gland; (ii) inhibiting the production of androgens by gonadal tissue via a negative feedback loop on pituitary gonadotrophin release; and (iii) regulating genes that suppress sebaceous gland growth or lipid production (25).

Inflammation

The microcomedo will continue to expand with densely packed keratin, sebum, and bacteria. Eventually this distension will cause follicular wall rupture. The extrusion of the keratin, sebum, and bacteria into the dermis results in a brisk inflammatory response. The predominant cell type within 24 hours of comedo rupture is the lymphocyte. CD4+ lymphocytes are found around the pilosebaceous unit while CD8+ cells are found perivascularly. One to two days after comedo rupture, the neutrophil becomes the predominant cell type surrounding the burst microcomedo (26).

Propionibacterium Acnes

As mentioned above, *P. acnes* also plays an active role in the process of inflammation. *P. acnes* is a gram-positive, anaerobic, and microaerobic bacterium found in the sebaceous follicle. Adolescents with acne have higher concentrations of *P. acnes* compared to non-acne controls. However, there is no correlation between the raw number of *P. acnes* organisms present in a sebaceous follicle and the severity of the acne (27).

The cell wall of *P. acnes* contains a carbohydrate antigen that stimulates antibody development. Those patients with the most severe acne have the highest titers of antibodies (28). The anti-*P. acnes* antibody enhances the inflammatory response by activating the complement cascade and thus initiating pro-inflammatory events (29). *P. acnes* also facilitates inflammation by eliciting a delayed type hypersensitivity response (30) and by producing lipases, proteases, hyaluronidases, and chemotactic factors (31). Additionally, *P. acnes* has been shown to stimulate an upregulation of cytokines by binding to toll-like receptor 2 (TLR-2) on monocytes and polymorphonuclear cells surrounding the sebaceous follicle (32). After binding TLR-2, pro-inflammatory cytokines such as IL-1, IL-8, IL-12, and TNF-alpha are released (33,34).

The four elements of acne pathogenesis—follicular keratinocyte hyperproliferation, seborrhea, inflammation, and *P. acnes*—are intertwined steps in the formation of acne. Various acne treatments target different elements in acne pathogenesis. Understanding the mechanisms of action of the multitude of therapeutic options in treating acne will help assure better therapeutic results.

MORPHOLOGY

Clinically, acne can present as non-inflammatory or inflammatory lesions or both. Non-inflammatory acne is marked by the presence of codemos. Codemos are follicular-based papules that may be either open or closed. Open codemos, commonly called blackheads, are papules with prominent dilated follicular ostia. Closed codemos, or whiteheads, are flesh colored papules without an evident follicular opening. Inflammatory acne can include lesions such as erythematous papules, pustules, nodules, cysts, or plaques. Post-inflammatory hyper- or hypopigmentation are common sequelae of inflammatory acne. However, scarring can be a complication of both inflammatory and non-inflammatory acne.

TOPICAL RETINOID

All acne lesions begin as non-inflammatory lesions, either open or closed codemos. Topical retinoids are a mainstay of acne treatment due to their ability to hamper the primary acne lesion, the microcodemo. Additionally, retinoids have fairly potent anti-inflammatory effects. Retinoids are structural and functional analogs of vitamin A that exist in both topical and systemic forms. They act by binding to two nuclear receptor families within keratinocytes: the retinoic acid receptors (RAR), and the retinoid X receptors (RXR). Each of the receptor families contains three receptor isotypes: alpha, beta, and gamma. In the human epidermis RXR receptors are by and large the alpha isotype and RAR are generally the gamma isotype (35). Both families of receptors act as ligand-activated transcription factors. The RAR receptors function as a heterodimer by binding to RXR. The RXR receptors may act as homodimers or may bind to other nuclear receptors such as: vitamin D3, thyroid hormone, and peroxisome proliferator-activated receptors. The retinoid receptors bind to specific regulatory DNA sequences called retinoid hormone response elements (HREs) where transcription is activated. The retinoid HREs activate the transcription of genes that normalize follicular keratinization, and decrease cohesiveness of keratinocytes. This prevents the formation of microcodemos. The retinoid-receptor complex also antagonizes genes that do not contain retinoid HRE. AP-1 and NF-IL6 are key transcription factors in inflammatory responses. The retinoids suppress these transcription factors by competing for the co-activator proteins needed to activate AP-1 and NF-IL6 (36). These combined anti-comedogenic and anti-inflammatory properties make retinoids beneficial for patients with either non-inflammatory or inflammatory acne.

Tretinoin

Tretinoin, the original topical retinoid, has been formulated in different vehicles in an attempt to decrease the irritation associated with the original formulations (Table 1). One delivery system involves the use of inert microspheres impregnated with tretinoin to allow for a slower delivery of tretinoin (Retin-A Micro® 0.04%, and 0.1% gel). Another formulation involves the combination of tretinoin with polyolprepolymer-2 (Avita®). Polyolprepolymer-2 prevents rapid percutaneous absorption of tretinoin, thus lessening some of the early irritation experienced with pure tretinoin products (37). Less peeling and

Table 1 Topical Retinoid Preparations Used for Treatment of Acne Vulgaris

Drug	Trade	Vehicle	Concentration
Tretinoin	Retin-A	Cream	0.025%, 0.05%, 0.1%
		Gel	0.01%, 0.025%
		Solution	0.05%
	Retin-A Micro	Gel with microsponge system	0.04%, 0.1%
	Avita	Cream	0.025%
		Gel	0.025%
	Altinac	Cream	0.025%, 0.05%, 0.1%
	Renova	Cream	
	Generic	Cream	0.025%, 0.05%, 0.1%
		Gel	0.025%
Adapalene	Differin	Cream	0.1%
		Gel	0.1%
		Solution	0.1%
Tazarotene	Tazorac	Gel	0.05%, 0.1%
		Cream	0.05%, 0.1%

Many topical retinoids are available in different vehicles and varying concentrations.
Source: Adapted from Ref. 11.

drying can be seen in patients using Avita® (37,38). A multi-center, double-bind, parallel study demonstrated comparable efficacy between Avita® and tretinoin 0.025% cream in 215 patients after 12 weeks of treatment (38).

Adapalene

Adapalene and tazarotene are topical medications that are formulated to bind the to the RAR without affinity for the RXR. Adapalene is a naphthoic acid derivative that was manufactured to be structurally similar to a naturally occurring hormone, retinoic acid. It works by directly binding to the RAR gamma and beta. A multi-center trial comparing adapalene 0.1% gel to tretinoin 0.025% gel found adapalene to produce a greater decrease in inflammatory and non-inflammatory lesions over a 12-week period. The adapalene group also had significantly less side effects of erythema, scaling, dryness, and burning (39). Adapalene is light stable allowing for daytime use. Adapalene is also resistant to oxidation by benzoyl peroxide. It is available in 0.1% concentration in a cream, solution, pledget, and propylene glycol-based gel.

Tazarotene

Tazarotene, a synthetic retinoid, exerts it action through its metabolite tazarotenic acid that binds RAR-beta and gamma. Studies have shown that tazarotene 0.1% gel is more effective than tretinoin 0.025% gel (40) or tretinoin 0.1% microspheres (41). Tazarotene can be used once daily overnight similarly to tretinoin or it can be applied for a brief period, and then washed off. This latter method minimizes irritation but maintains efficacy by exposing the skin to the retinoid for only five minutes once a day (42). It is available in 0.05% or 0.1% cream or gel formulations.

While the other topical retinoids are classified as pregnancy category "C," tazarotene is category "X" (Table 2). In two surveys of patients with first trimester exposures to tretinoin, there was no increased incidence of congenital malformations

Table 2 Comparison of Topical Retinoids

Generic	Required nighttime use?	Inactivated by benzoyl peroxide?	Pregnancy category
Tretinoin	Yes	Yes	C
Adapalene	No	No	C
Tazarotene	No	Yes	X

Comparison of topical retinoids with regards to inactivated by sunlight and benzoyl peroxide and their pregnancy categories.

(43,44). In one study, six patients who inadvertently became pregnant while on tazarotene had no babies with congenital malformations (45). This difference in categorization is due to the dual indication for tazarotene for both acne vulgaris and psoriasis. In psoriasis patients, larger amounts of tazarotene are used thus raising plasma levels of the retinoid to teratogenic potential. Only one pregnancy class can be assigned to a drug, therefore category "X" was designated given thus drug's potential to be used on a large surface area (46). Therefore, female patients must undergo contraceptive counseling while on tazarotene. For women who intend to become pregnant, there is no specific recommended wash-out period after tazarotene use (45).

Adverse Effects

Although effective for different types of acne, topical retinoids commonly cause adverse effects. These are generally mild in severity and usually during the start of therapy. Within the first month of treatment many patients experience a "retinoid dermatitis." This may consist of erythema, burning, scaling, pruritus, and dryness. These effects tend to decrease with continued use. Detailed instructions on appropriate use of retinoids can help limit any adverse side effects and enhance tolerability (Table 3). In general, a pea-sized amount should be applied evenly over the entire face. If any medicine is visible on the skin after application too much was applied and additional irritation may ensue. Instructing patients to apply their retinoid to dry skin can also minimize retinoid dermatitis. Patients should be advised to wait 15 minutes after washing the face to apply a topical retinoid. Wet skin enhances the penetration of the retinoid into the dermis, thus exacerbating irritation. A gradual increase in application frequency can also help to minimize irritation. The patient should apply the retinoid starting every other night or every third evening for the

Table 3 Patient Information About Topical Retinoids

When initiating treatment, apply topical retinoids every second or third day for the first couple weeks and gradually increase to once-daily application
Tretinoin must be applied at night
Wait 15 minutes after washing face to apply the retinoid
Apply a pea-sized amount to cover the entire face
Do not use a benzoyl peroxide product at the same time of day as retinoid application (concomitant use all right with adapalene)
Redness, burning, scaling, pruritus, and dryness may occur, especially during the first month of treatment. These side effects generally decrease with use
A non-comedogenic moisturizer may be used to prevent dryness
Photosensitivity may occur with all retinoids
Some patients experience a flare of their acne during the first few weeks of treatment

The above patient information regarding topical retinoid use can improve compliance and efficacy.

first one to two weeks of treatment. The patient can then gradually increase the frequency to nightly use as tolerated. Tolerance is often achieved in three to four weeks. It is important that the topical retinoid be applied at night-time for two reasons. Firstly, patients who use topical retinoids during the daytime notice increased sensitivity to ultraviolet (UV) light. Secondly, tretinoin is unstable when exposed to sunlight. When exposed to light, 50% of tretinoin is degraded in two hours (47). The synthetic formulations adapalene (47) and tazarotene (48) remain chemically stable when exposed to sunlight, and may be applied morning or evening. To combat irritation it is recommended that a non-comedogenic facial moisturizer be applied during the daytime. Some patients experience a pustular acne flare during the initial weeks of retinoid treatment that subsides with use. Patients should be warned of this possibility and encouraged to continue treatment through the exacerbation.

CLEANSERS

Treatment with retinoids, and acne itself, will cause dysfunction of the skin barrier. Facial cleansers also interact with proteins and lipids on the stratum corneum, and may further disrupt the skin barrier. However, acne patients need to use cleansers to control the level of skin oils and microbial levels. It is important to utilize facial cleansers that minimally disrupt the stratum corneum so that the barrier can be preserved. Soaps are alkaline cleansers that increase the skin's normal pH causing a decrease in the cutaneous lipid content (49). Soaps that contain antibacterial agents (such as triclosan) can inhibit gram-positive cocci but increase gram-negative rods (50). The irritant effects of soaps is worsened by hard water (51). Rather than using a soap, patients should cleanse their face with a syndet (synthetic detergent) cleanser. Rather than being alkaline, syndets have a pH close to the skin's pH of 5.5 (Table 4). Syndets used with hard water do not produce a scum on the skin, as do soaps. Syndets are minimally irritating, and compatible with other acne treatment regimens (52,53). Patients using syndets report more improvement in their acne than those using soaps. Syndets will also aid in minimizing irritation from other acne treatments such as tretinoin (53).

There are cleansers other than syndets may be more irritating but contain superior anti-acne properties. These other products, such as hydroxy acids and benzoyl peroxide, may exist as washes, and also as creams, gels, scrubs, and peels.

HYDROXY ACIDS

Hydroxy acids are present in over-the-counter and prescription formulations (Table 5). Alpha-hydroxy acids, such as glycolic acid and lactic acid, are water-soluble, and therefore penetrate to the dermis. Glycolic and lactic acids are derived from sugar cane and sour milk, respectively. Beta-hydroxy acids, such as salicylic acid, are lipid-soluble, and penetrate into the upper epidermis and into the pilosebaceous unit. Salicylic acid is derived from willow bark, wintergreen leaves, and sweet birch, and is also available in synthetic forms (54,55). Both alpha- and beta-hydroxy acids decrease cohesion among the keratinocytes in the stratum corneum, causing exfoliation (56,57). Due to their ability to penetrate the pilosebaceous unit, beta-hydroxy acids such as salicylic acid have a stronger comedolytic effect than alpha-hydroxy acids (58). However, in comparison with tretinoin and isotretinoin, salicylic acid is a mild comedolytic agent. Salicylic acid is available in both over-the-counter and prescription preparations ranging from 0.5 to 5% (59). Over-the-counter products are listed in Table 6.

Table 4 Cleansers

Brand name	pH	Composition
Aderm	6.44	Syndet
Avecyde	3.61	Syndet
Avéne	6.94	Syndet
Cetaphil	7.72	Syndet
Dove white	7.53	Syndet
Dove baby	7.0	Syndet
Dove (liquid)	5.16	Syndet
Dove pink	7.23	Syndet
Johnson's baby	11.9	Soap
Johnson's baby oat	12.35	Soap
Lux with glycerin	12.38	Soap
Nivea baby creamy	12.35	Syndet[a]
Nivea bath care	12.21	Syndet[a]
Nivea bath c. Almond	12.22	Syndet[a]
Nivea bath c. Oat	12.30	Syndet[a]
Oilatum	10.26	Syndet[a]
Natural oilatum	10.01	Syndet[a]
Zest neutral	9.85	Soap
Zest citrus sport	9.75	Soap
Zest herbal	9.97	Soap
Zest aqua	9.89	Soap
Palmolive green	10.18	Soap
Palmolive (white)	10.23	Soap
Palmolive botanicals	10.38	Soap
Camay classic	10.38	Soap
Camay gala	10.36	Soap
Camay soft	10.26	Soap
Rosa venus	10.65	Soap

pH and composition of some commercially available cleansers. The pH of each emulsion or liquid cleanser was recorded by using the Chemcadet pH meter (Cole-Parmer Instrument Co.).
[a] Plus mineral oil.
Source: Adapted from Ref. 60.

Table 5 Properties of Hydroxy Acids

Hydroxy acid	Solubility	Derived from	Penetration	Action
Alpha-hydroxy acids	Water-soluble		Dermis	Exfoliative
Glycolic acid		Sugar cane		
Lactic acid		Sour milk		
Beta-hydroxy acid	Lipid-soluble		Epidermis & pilose baceous unit	Exfoliative & comedolytic
Salicylic acid		Willow bark, wintergreen leaves, sweet birch		

Solubility, derivation, penetration, and action of alpha- and beta-hydroxy acids.

BENZOYL PEROXIDE

Benzoyl peroxide is a topical medication that has bactericidal effects to reduce *P. acnes*. It is available in both over-the-counter (Table 7) and prescription formulations as a bar soap, wash, gel or lotion in varying concentrations. The stay-on formulations of benzoyl peroxide will decrease *P. acnes* counts more so than the washes although both significantly decrease *P. acnes*. The concomitant use of benzoyl peroxide with antibiotics will lessen *P. acnes* resistance to antibiotics and increase the bactericidal effect of the antibiotic (61). None of the topical antibiotics alone is more effective against *P. acnes* than benzoyl peroxide (27). Benzoyl peroxide products need to be used at different times of the day than tretinoin or tazarotene. Oxidation of these retinoids, and thus decreased efficacy, can occur when in contact with benzoyl peroxide. A benzoyl peroxide product may be utilized in the morning with night-time application of a retinoid. Caution should be given to the patient that benzoyl peroxide products can bleach linens and clothing. Benzoyl peroxide allergic contact dermatitis may happen but is rare with a 1:500 incidence (62).

There are several topical products that combine benzoyl peroxide with either erythromycin or clindamycin. These combination topical products treat inflammatory acne better than either product alone (63,64). The shelf-life for these combination products is limited; therefore, some formulations of erythromycin, and benzoyl peroxide need to be refrigerated. Diarrhea and pseudomembranous colitis are rare but have been associated with topical clindamycin.

OTHER TOPICAL TREATMENTS

Other topical products with antimicrobial effects include azelaic acid and sodium sulfacetamide. Azelaic acid is a naturally occuring nine-carbon dicarboxylic acid. It is available for the treatment of acne in a 20% cream preparation. Azelaic acid has antimicrobial and weak anti-comedogenic effects. It reduces the production of keratohyalin granules in the pilosebaceous epithelium, thus normalizing ductal keratinocyte proliferation (62). Its anti-microbial effect is inferior to that of antibiotics or benzoyl peroxide (65). Azelaic acid can also decrease pigmentation by competing with tyrosinase (66). It may therefore be helpful for acne patients with post-inflammatory hyperpigmentation. It causes minimal erythema and does not produce the same degree of irritation as topical retinoids (62). Additionally, azelaic acid is safe for use in pregnancy.

Sulfur–sodium sulfacetamide is another well-tolerated topical antimicrobial. It is available as washes, bars, and creams. Sulfur presumably inhibits the growth of *P. acnes* by inhibiting its sustenance, para-aminobenzoic acid (PABA). The irritant effect on the skin causes keratolysis. The addition of sodium sulfacetamide lotion to sulfur has made its use more cosmetically acceptable.

ORAL ANTIBIOTICS

Oral antibiotics are often administered to patients with moderate to severe acne or in patients in whom topical therapy has failed. Patients with moderate acne with scarring or those whose acne covers a large surface area making topical application difficult may also be candidates for oral antibiotics. Oral antibiotics used in acne are typically of the tetracycline or macrolide family (Table 8).

Tetracyclines

The tetracyclines work by interacting with the 30S ribosomal subunit of bacteria, and thus inhibiting protein synthesis. Included in the tetracycline family are tetracycline,

Table 6 Over-the-Counter Salicylic Acid Products

Product	Strength (% salicylic acid)
Aveeno Clear Complexion Cleansing Bar	1%
Aveeno Clear Complexion Correcting Treatment	1%
Aveeno Clear Complexion Daily Cleansing Pads	0.5%
Biore Pore Perfect Blemish Fighting Cleansing Cloths	0.5%
Biore Pore Perfect Unclogging Scrub	2%
Biore Pore Perfect Warming Anti-blackhead Cream Cleanser	2%
Bye Bye Blemish Anti-acne Serum	1%
Bye Bye Bleamish Anti-acne Cleanser	0.5%
Clean and Clear Acne Wash Oil-free	2%
Clean and Clear Advantage Acne Spot Treatment	
Clean and Clear Advantage Acne Cleanser	2%
Clean and Clear Advantage Daily Cleansing Pads	2%
Clean and Clear Blackhead Clearing Scrub	2%
Clearasil 3 in 1 Acne Defense Cleanser	2%
Clearasil Icewash Gel Cleanser	2%
Clearasil Pore Cleansing Pads	2%
Clearasil Wipes	2%
Cuticura Acne Treatment Foaming Face Wash	
Eucerin Clear Skin Formular Concealer Pencil	
Neutrogena Acne Wash Foam Cleanser	2%
Neutrogena Acne Wash Cloths	2%
Neutrogena Acne Wash Cream Cleanser	2%
Neutrogena Blackhead Eliminating Daily Scrub	2%
Neutrogena Body Clear Body Wash	2%
Neutrogena Oil-free Acne Wash	2%
Neutrogena Oil-free Acne Wash Cream Cleanser	2%
Neutrogena Oil-controlling Cleansing Pads	2%
Neutrogena Healthy Skin Anti-wrinkle Anti-blemish Cleanser	0.5%
Neutrogena Advanced Solutions Acne Mark Fading Peel	2%
Neutrogena Deep Clean Cream Cleanser	
Neutrogena Clear Pore Treatment	2%
Olay Daily Facials Clarity Foaming Cleanser	2%
pH Isoderm Facial Wash Clear Confidence	2%
Stridex Face Wipes to Go	0.5%
Stridex Triple Action Acne Pads	2.0%
Stridex Sensitive Skin Pads	0.5%

Some commercially available over-the-counter salicylic acid products and the percent of salicylic acid they contain.

minocycline, and doxycycline. In addition to their antimicrobial effect, all of the tetracyclines are anti-inflammatory agents. They inhibit white blood cell chemotaxis, decrease lipase production by *P. acnes,* and decrease cytokine production. They also offer anti-inflammatory effects by decreasing the activity of matrix metalloproteinases (MMPs) (67). MMPs degrade several components of the extracellular matrix.

Tetracycline is a first-generation tetracycline. It is often administered at 500 mg twice daily for acne. Tetracycline should not be taken with milk as calcium blocks its absorption in the gut. It therefore must be taken on an empty stomach, one hour prior to or two hours after meals. Tetracycline may also cause gastrointestinal upset in some patients. Patients should also be warned of increased photosensitivity while on tetracycline. Other

Table 7 Over-the-Counter Benzoyl Peroxide Products

Product	Strength (% benzoyl peroxide)
Clean and Clear Continuous Control Acne Cleanser	10%
Clean and Clear Persa-gel Maximum Strength	10%
Clearasil Cream	10%
Clearasil Ultra Acne Treatment Cream	10%
Neutrogena Clear Pore Cleanser/Mask	3.5%
Neutrogena On-the-Spot Acne Treatment Vanishing Formula	2.5%
Oxy Acne Wash	10%
Oxy Acne Treatment Vanishing	10%
Panoxyl Bar	10%
Panoxyl Aqua Gel	10%
Stridex Power Pads	2.5%
Zapzyt Bar	10%
Zapzyt Treatment Gel	10%

Some commercially available over-the-counter benzoyl peroxide products and the percent of benzoyl peroxide they contain.

photosensitive side effects that may occur while on tetracycline include painful photo-onycholysis and pseudoporphyria. Tetracycline is deposited in areas of calcification. As a result, hyperpigmentation of deciduous and permanent teeth and bone may occur. For this reason, tetracycline should not be used in children under the age of 10 as deposition in the bone epiphyses may halt bone growth. Tetracycline is pregnancy category D since it can be deposited in the fetal bones. Nursing mothers should not be given tetracyclines due to the potential for drug excretion through the breast milk.

Doxycycline is a second generation tetracycline administered at 100 mg twice daily for acne. It is better absorbed from the gastrointestinal tract than tetracycline, and can be taken with food, although maximum absorption occurs when taken 30 minutes prior to a meal. Like tetracycline, it can be deposited in areas of calcification such as the teeth and bones, and therefore cannot be used in children under the age of 10, and is pregnancy category D. Photosensitivity is most common with doxycycline and is dose-dependent. 42% of patients taking a total of 200 mg a day will develop photosensitivity (68).

Doxycycline can also be administered for acne at subantimicrobial doses of 20 mg twice a day. In this manner the doxycycline is given at a low dose so that it has only anti-inflammatory effect, and not an antimicrobial effect. Without an antimicrobial action, there

Table 8 Comparing the Oral Tetracylines

Antibiotic	Dosing	Advantages	Disadvantages
Tetracycline	Taken on an empty stomach without milk	Inexpensive	Gastrointestinal upset
Minocycline	May be taken with food	Rapid onset of action	Risk of pigmentation, dizziness, autoimmune disorders
Doxycycline	May be taken with food	May be used at subantimicrobial doses	Photosensitivity

Comparing tetracycline, minocycline, and doxycycline with regards to dosing and the unique advantages and disadvantages of each.

is no opportunity for antibiotic resistance to arise. Doxycycline is the almost effective of the tetracyclines used at subantimicrobial doses because it is the most potent inhibitor of MMP (69). In a study of 40 acne patients who received doxycycline 20 mg po bid for 6 months, no adverse events such as nausea, vomiting, phototoxicity, or vaginitis were noted (70).

Minocycline is another second generation tetracycline given at 100 mg twice daily. Of the tetracyclines it has the best gastrointestinal absorption. It can be taken with food but is best absorbed 30 minutes prior to a meal. Compared to tetracycline and doxycycline, minocycline has more rapid clinical improvement. It also demonstrates a more persistent reduction of inflammation. In vitro, minocycline has the greatest reduction of *P. acnes* of all the antibiotics used for acne (65). Minocycline's superior effects are due to its high lipophilicity, and thus better penetration into the pilosebaceous unit. Minocycline can potentially cause a blue-grey hyperpigmentation, vestibular disturbances, or a hypersensitivity drug reaction (70). Three types of hyperpigmentation can occur. Type I hyperpigmentation occurs in areas of scar tissue. Type II hyperpigmentation occurs on previously normal skin, commonly on the anterior shins. Type III hyperpigmentation has a predilection for sun-exposed areas, and often is a diffuse hyperpigmentation. Since minocycline is highly lipophilic it can easily cross the blood-brain barrier. This may result in vestibular disturbances such as dizziness, vertigo, or ataxia. Rarer side effects of minocycline include drug-induced lupus, serum sickness, hepatic failure, and vasculitis. With the exception of serum sickness (which on average occurs 16 days after starting therapy), these side effects often occur after more than a year of therapy (71).

Benign intracranial hypertension, also known as pseudotumor cerebri, can occur with any of the tetracycline antibiotics, and is an increase in cerebrospinal fluid. This increase in intracranial pressure is seen most frequently with minocycline due to its ability to cross the blood-brain barrier. Pseudotumor cerebri can occur between four weeks and 18 months after starting therapy. Patients will complain of a headache that worsens in the evening, diplopia on lateral gaze, and nausea. Papilledema will be demonstrated by ophthalmologic examination. A lumbar puncture can aid in diagnosis, and also be therapeutic by relieving pressure of excess cerebrospinal fluid.

Macrolides

The macrolide antibiotics can also be useful in treating acne. This family of antibiotics works by binding irreversibly to the 50S ribosomal subunit thus inhibiting translocation during protein synthesis. Erythromycin and clindamycin are members of the macrolide family that are commonly used in acne. Both are used alone or in conjunction with benzoyl peroxide as a combination product. When given orally, erythromycin is administered at 500 mg twice daily. Erythromycin can and should be taken with food as it commonly causes gastrointestinal upset. It is safe in pregnancy and in lactating women, although erythromycin estolate should be avoided in these groups as it may cause cholestatic jaundice. Erythromycin inhibits the cytochrome P450 system thus causing reduced clearance of theophylline, warfarin, carbamazepine, and cyclosporine (70,72). Concomitant use of these medications should be avoided.

Clindamycin

The oral use of clindamycin is limited since 20–30% of patients will experience diarrhea. Oral clindamycin can also cause an overgrowth of *Clostridium difficile* in the gut thus causing pseudomembranous colitis (72). Topical clindamycin is generally well tolerated, and is available by prescription in solution, gel, and foam formulations.

Trimethoprim/Sulfamethoxazole

Trimethoprim/sulfamethoxazole is another oral antibiotic option for acne. It is administered 160 mg/800 mg twice daily. It works by inhibiting dihydrofolate reductase thus impeding purine and pyrimidine synthesis. It has a broad spectrum of antimicrobial activity, and therefore should be reserved as a second line agent or for patients with gram-negative folliculitis. Due to its sulfa moiety, trimethoprim/sulfamethoxazole also has the potential to cause a drug hypersensitivity syndrome with multi-organ involvement. Anemia, thrombocytopenia, and agranulocytosis are also potential side effects of trimethoprim/sulfamethoxazole.

Antibiotic Counseling

It is important to discuss and educate patients on the potential side effects of each antibiotic in order to maximize patient compliance. Patients may express several concerns about oral antibiotic use. One of these concerns may be possible reduced efficacy of their oral contraceptives. No pharmacokinetic interaction has been demonstrated between oral contraceptive pill and antibiotics (except rifampin). Oral contraceptive failure rates while on antibiotics, including those used for treating acne, fall within the range of oral contraceptive failure rates of patients not on antibiotics which is 1% to 3% (73). There are some individuals who will have decreased absorption of the oral contraceptive due to changes in the gut flora by antibiotics. It is impossible to predict who these patients will be. Therefore, all patients should be counseled regarding the small risk that their oral contraceptive may be less effective while taking antibiotics. Patients may also express concern regarding the publicized cancer risk with antibiotic use. A study in 2004 showed an increased risk of breast cancer with long-term antibiotic use (74). This risk was the same if antibiotics were being used to treat acne/rosacea or respiratory tract infections. A direct effect was not demonstrated in this study, only an association. It is important to point out to patients that no causal relationship between antibiotic use, and breast cancer was identified in this study.

Frequently antibiotics produce favorable results but sometimes a patient does not respond to antibiotic treatment. Several reasons exist for a poor clinical response. The antibiotic may have been given at an inadequate dose or for an inadequate duration. A maximum response is usually seen in three to four months. The patient may have been given suboptimal instructions on use or had poor compliance. Patients with a high sebum excretion rate (greater than 2.5 micrograms/cm/minute) may not respond due to dilution of the antibiotic in the pilosebaceous unit. Antibiotics may not be helpful if the patient is misdiagnosed with acne when the eruption truly is folliculitis due to gram negative enterobacteria, staphylococci, or yeasts.

Antibiotic Resistance

A patient may also not respond to antibiotics therapy if there is *P. acnes* resistance (75). Antibiotic resistance is a real problem of growing concern. The overall incidence of *P. acnes* antibiotic resistance increased from about 20% in 1978 to approximately 62% in 1996. Resistance of *P. acnes* is most common for erythromycin, clindamycin, tetracycline, doxycycline, and trimethoprim. Minocycline resistance is present in about 1% of patients today (76). There is no resistance to benzoyl peroxide, azeleic acid, or sulfur.

Several things can be done to minimize selection, and spread of antibiotic resistant strains of *P. acnes*. Antibiotics should be used judiciously, and only until control is achieved. The antibiotic should then be discontinued. If repeat treatment with antibiotics is required, the same antibiotic should be reused (unless it has lost efficacy). Patients who

are on oral or topical antibiotics should also use a benzoyl peroxide product to reduce the numbers of antibiotic resistant organisms. Oral, and topical antibiotics with dissimilar properties should not be used concomitantly, as combining these agents may cause cross resistance. Patient education is important to maximize compliance and prevent resistance.

HORMONAL THERAPY

Hormonal therapy is another treatment option for females with acne. Hormonal therapy may be helpful in acne patients with hirsutism, irregular menstrual periods and/or other signs of hyperandrogenism. Patients who have failed isotretinoin or oral antibiotics may still benefit from hormonal therapy. Women with flares of acne around the time of menses and those with excessive seborrhea may also respond well to hormonal therapy. Only oral retinoids and hormonal therapy can decrease sebum production. Anti-androgens, glucocorticoids, and oral contraceptives are all types of hormonal therapies (77).

Spironolactone

The anti-androgens include the androgen receptor blockers spironolactone, cyproterone acetate, and flutamide. Spironolactone is an aldosterone antagonist that also blocks the androgen receptor (72). Oral spironolactone reduces sebum excretion by 45–50%. It is taken 25–100 mg orally twice a day. Patients frequently experience breast tenderness and menstrual irregularities with spironolactone. These side effects increase with the dosage of spironolactone, and can be minimized by using an oral contraceptive. If a woman becomes pregnant with a male fetus, spironolactone as an anti-androgen may lead to feminization of the male genitalia and endocrine dysfunctions (78). Spironolactone can also cause mild elevations of potassium, and should therefore be used with caution in patients with renal or cardiac disease (79,80). For patients taking ethinyl estradiol/drospirinone (Yasmin®) in conjunction with spironolactone, this risk of hyperkalemia is increased.

Cyproterone Acetate

Cyproterone acetate is a hydroxy-progesterone that blocks the binding of androgens to their receptors. It also inhibits the synthesis of androgens in the adrenal glands and in the skin. Cyproterone acetate is not available in the United States but is available in Canada and Europe. It is incorporated in an oral contraceptive pill at 2 mg per day with ethinyl estradiol. Women with abnormal androgen metabolism can take additional 10–50 mg once daily during the first 10 days of their menstrual cycle (81).

Flutamide

Flutamide is a potent anti-androgen that is used to treat prostate cancer, and can also be useful for acne (82). It is administered at 250 mg twice daily in combination with an oral contraceptive. Liver function tests must be monitored in patients on flutamide as it can cause fatal hepatitis (83). Patients with adrenal hyperandrogenism may benefit from a combination of low dose glucocorticoidsteroid with an oral contraceptive. The glucocorticoid works byblocking adrenal androgen production. Prednisone 2.5–5 mg can be given daily at bedtime. Alternatively, dexamethasone 0.25–0.75 mg can be given nightly. Chronic glucocorticoid use may cause adrenal suppression (especially dexamethasone), and should be used with caution (84).

Oral Contraceptive

Oral contraceptives have several mechanisms of action in treating acne. They reduce androgen production by both the ovaries and the adrenal glands. Oral contraceptives also reduce free serum testosterone by increasing sex hormone-binding globulin. Sebum production is decreased with oral contraceptive use, possibly due to androgen reduction or possibly directly due to increased levels of estrogens (77). Most formulations consist of an estrogen in combination with a progestin. Progestin is needed to minimize the increased risk of endometrial cancer with unopposed estrogens. Progestins themselves have intrinsic androgenic activity, which can worsen acne in addition to causing changes in lipid metabolism and glucose intolerance (85). The newer generations of progestins (second and third generations) have been formulated to have lower androgenic properties. The second-generation progestins include ethynodiol diacetate, norethindrone, and levonorgesterol. The third-generation progestins have less cross reactivity with the androgen receptor, and increase sex hormone binding globulin. These oral contraceptives that lessen androgenic activity include: desogestrel, norgestimate, and gestodene. Only two oral contraceptives are currently FDA-approved for the treatment of acne, Ortho Tri-Cyclen® (Ortho, Raritan, NJ) and Estrostep® (Parke Davis Laboratories, Detroit, MI). Ortho Tri-Cyclen® is a triphasic contraceptive contaning ethinyl estradiol (35 micrograms) and the third-generation progestin, norgestimate. Estrostep® contains ethinyl estradiol (graduated dose from 20–35 micrograms), and the second-generation progestin, norethindrone acetate (86).

Clinical improvement due to hormonal therapy will be evident as early as three months. Hormonal therapy is especially beneficial for acne in a "beard" distribution, involving the mandible and chin. Co-management with a patient's gynecologist is needed so that patients may be appropriately followed while on an oral contraceptive. Often times, the oral contraceptives approved for acne may not be the best choice for patients experiencing heavy or abnormal menses. Relatively common side effects from oral contraceptives includes: nausea, vomiting, abnormal menses, weight gain, and breast tenderness. More detrimental but rarer side effects include: thrombophlebitis, pulmonary embolism, and hypertension (87). The risk of these serious side effects increases in patients who smoke cigarettes.

ISOTRETINOIN

Isotretinoin (13-cis retinoic acid) is an oral retinoid that has been available for the treatment of acne since 1971 in Europe, and since 1983 in the United States. The Food and Drug Administration (FDA) indication for the use of isotretinoin is severe, nodulocystic acne that has not been helped by other treatments, including antibiotics. However, patients with other forms of acne have also benefited from isotretinoin. These patients include those with: significant acne unresponsive to treatment including oral antibiotics, acne that results in significant physicial or emotional scarring, and patients with gram-negative folliculitis, pyoderma faciale, and acne fulminans (88,89).

The exact mechanism of action of isotretinoin is not known. It is believed that 13-cis-retinoic acid exerts its action by isomerization to all-trans-retinoic acid, which then interacts with the retinoid receptors (90). It is the only acne medicine available that affects all four pathogenic factors of acne. Isotretinoin is comedolytic, reduces sebaceous gland size (up to 90%), and suppresses sebum production which in turn inhibits *P. acnes* and its ability to elicit inflammation (91). During the course of isotretinoin therapy, the pustular lesions generally clear first, and the codemos will be the last to resolve. Lesions on the face and upper arms tend to respond faster to isotretinoin than lesions on the trunk (92).

Dosing

There is variation in the dosing of isotretinoin with daily doses ranging from 0.1 to 2 mg/kg. Typically, 0.5–2 mg/kg/day is recommended for 16–20 weeks to reach a total cumulative dose of 120–150 mg/kg. A lower starting dose may be necessary in patients with significant inflammatory acne to prevent flares in the first month of treatment. Patients at risk for initial flaring may be concomitantly given prednisone to reduce flaring and prevent exuberant granulation tissue. Patients with pyoderma faciale should be controlled on prednisone prior to beginning isotretinoin.

Studies have demonstrated that about 23% of patients using the typical dosing may need to repeat treatment with isotretinoin. 96% of patients who experience a relapse of their acne do so within the first three years. Of patients taking a lower regimen of 0.1 mg/kg/day, 40% need a repeat course (93). Factors contributing to the need for additional courses are lower dose regimens (0.1–0.5 mg/kg/day or cumulatively less than 120 mg/kg), the presence of severe acne, being a female older than 25 years at the start of therapy, and having a prolonged history of acne (94,95). If repeat therapy is needed, at least two to three months should elapse between courses. An even longer interval may be sensible as the effects of isotretinoin can be seen five months after discontinuation.

Side Effects

Since RAR are found in many organs of the body, isotretinoin can cause numerous side effects (Table 9). This profile of adverse effects closely mimics those of hypervitaminosis A (96). Mucocutaneous side effects are the most common and are dose dependent. The most frequently encountered mucocutaneous side effects are: cheilitis, generalized xerosis, and dry mucosa. Cheilitis is so common that its absence would indicate a suboptimal dose or cause suspicion of the patient's compliance. Dry nasal mucosa frequently results in epistaxis (97). Xerophthalmia is common with subsequent contact lens intolerance, and possible conjunctivitis. Photophobia, decreased night vision, keratitis, and optic neuritis are less common, while cataracts, and corneal opacities rarely develop. Hair thinning, and hair loss occurs in less than 10% of patients on isotretinoin (98,99).

Neuromuscular complaints are relatively common in patients taking isotretinoin. About 14% of patients will experience myalgias, and a higher percentage will experience arthralgias and back pain. These neuromuscular complaints may coexist with a transient rise in creatinine phosphokinase, and are more common in physically active patients (100,101).

Nausea, vomiting, diarrhea, and abdominal pain have occurred in patients on isotretinoin but are rare. Transient mild increases in liver transaminases occur in about 15% of patients. Frank hepatitis is very rare, and has been reported in adults but not in children on isotretinoin (102).

Pseudotumor cerebri, or benign intracranial hypertension, is a rare side effect of isotretinoin. Patients who develop this increase in intracranial pressure will complain of headache, blurred vision, double vision, and/or vomiting. If recognized by history, and the finding of papilledema, a lumbar puncture can confirm the diagnosis, and be therapeutic. The likelihood of developing pseudotumor cerebri is increased with concomitant tetracycline use (103).

The impact of isotretinoin on a patient's psychological well-being has incited much attention. From 1982 to May 2000, 37 cases of suicide, 110 cases of hospitalized depression, suicidal ideation, or suicide attempt, and 284 cases of nonhospitalized depression were reported to the FDA's Adverse Event Reporting System (104). In one population-based cohort study comparing isotretinoin users with oral antibiotic users, the relative risk for development of depression or psychosis was approximately 1.0, indicating

Table 9 Isotretinoin Side Effects

Teratogenicity
Hydrocephalus
Micorcephaly
External ear abnormalities
Microphthalmia
Craniofacial dysmorphia
Cardiac septal defects
Thymus gland abnormalities
Mucocutaneous
Chelitis[a]
Xerosis[a]
Skin fragility[a]
Palmoplantar peeling[a]
Dry nose[a]
Epistaxsis[a]
Pruritus[a]
Facial erythema/ rash[a]
Desquamation
Atrophy
Granulation tissue
Alopecia
Brittle nails
Acne fulminans
Pyogenic granuloma-like lesions
Ophthalmologic
Xerophthalmia[a]
Blepharitis
Papilledema
Blurred vision
Night blindness
Corneal opacities
Gastrointestinal
Nausea
Anorexia
Abdominal pain
Cirrhosis
Neuromuscular/Psychiatric
Headache[a]
Fatigue
Lethargy
Myalgias
Stillness
Irritiablility
Depression
Suicidal ideation
Psychosis
Papilledema
Pseudotumor cerebri
Rheumatologic
Arthralgias
DISH-like vertebral hyperostoses

(Continued)

Table 9 Isotretinoin Side Effects (*Continued*)

Altered bone remodeling
Extraspinal tendon and ligament calcification
Premature epiphyseal closure
Demineralization/ osteoporosis
Periosteal thickening
Laboratory
Elevated triglycerides[a]
Elevated cholesterol
Elevated liver function tests
Elevated creatine phosphokinase

[a] Denotes most common side effects.
Source: Adapted from Refs. 100, 129, 130.

no increased risk (105). A recent study demonstrated a decrease in depressive symptoms in patients undergoing treatment with isotretinoin (106). Further studies are needed to resolve this issue of causality.

Isotretinoin is a potent teratogen, and is rated pregnancy category X. The exact mechanism of embryopathy is unknown, but exposed infants have characteristic craniofacial defects as well as cardiac, thymus, and central nervous system abnormalities (107). Approximately 3–4 per 1000 women on isotretinoin become pregnant. In an effort to eliminate pregnancy while on isotretinoin, the manufacturer has implemented several regulations. There are additional consent forms for women regarding the potential teratogenicity. Appropriate contraception must be used one month prior to and one month following a course of isotretinoin. Two negative pregnancy tests must be obtained before starting isotretinoin, and a negative test must be obtained each month while on therapy.

Laboratory Monitoring

In addition to the laboratory tests that need to be performed to ensure that a woman is not pregnant, both men and women must get additional laboratory studies. A complete blood count, liver function test, and a lipid profile is checked at baseline and four weeks after starting isotretinoin. Elevated triglyceride levels occur in about 25–45% of patients (107). Those who have increased cholesterol during therapy often had elevated baseline cholesterol (108). Elevated liver function enzymes are also possible during isotretinoin but rarely does frank hepatitis develop. This elevation is reversible with discontinuation of isotretinoin. Other reversible changes during isotretinoin therapy include: leukopenia, thrombocytopenia, thrombocytosis, and an elevated erythrocyte sedimentation rate.

MANUAL TREATMENTS

In addition to topical and oral medications, physical modalities exist for treating acne. Comedone extraction can provide prompt cosmetic results. The keratinous debris of the open comedo may be extracted by using the Schamberg, Unna, and Saalfield types of comedo extractors. The closed comedo can also be removed by extraction but must first be nicked with an 18-gauge needle or an #11 blade. Extraction should not be attempted on an inflamed comedo or pustule due to the risk of scarring. Electrocautery and electrofulguration have also been reported as effective treatment for comedones. These means are often useful for treating large comedos, also known as macrocomedos. Macrocomedos are often resistant to topical retinoids.

Intralesional triamcinolone can be utilized to decrease both the size and pain of inflammatory cysts or nodules. Triamcinolone acetonide (2–5 mg/ml) is injected into these lesions using a 30-gauge needle. The steroid should be injected until the lesion blanches. The maximum amount of steroid used per lesion should not exceed 0.1 mL. Excess triamcinolone injected into a lesion may result in hypopigmentation, atrophy, telengiectasias, and needle tract scarring.

PHOTOTHERAPY

Various forms of phototherapy are under investigation for their use in treating acne vulgaris. Up to 70% of patients report that sun exposure improves their acne (109). This reported benefit may be due to camouflage by UV radiation-induced erythema and pigmentation, although it is likely that the sunlight has a biologic effect on the pilosebaceous unit and *P. acnes*. Porphyrins are formed endogenously by *P. acnes*, and are also acquired by exogenous sources. Protoporphyrin IX is taken up via cell wall receptors (110), and coproporphyrin III is the major endogenous porphyrin. Coproporphyrin III can absorb light at the near-UV and blue-light spectrum of 415 nm (111). In vitro irradiation of *P. acnes* with blue light leads to photoexcitation of endogenous bacterial porphyrins, singlet oxygen production, and subsequent bacterial destruction (112). Although UVB can also kill *P. acnes* in vitro, it is clinically insignificant since it has low skin penetration, and only high doses causing sunburn have be shown to improve acne (113,114). UV radiation may have anti-inflammatory effects by inhibiting cytokine action (115).

UV Radiation

Studies investigating the effect of UV radiation on acne have demonstrated a modest improvement in only inflammatory acne. There is some effect with UVB radiation alone, and slightly more benefit with combined UVB and UVA radiation. UVA light alone is the least beneficial. Twice-weekly phototherapy sessions are needed for clinical improvement. The therapeutic utility of UV radiation in acne is superseded by its carcinogenic potential (116–120).

Visible light is effective in treating both inflammatory and non-inflammatory acne lesions (121). A high-intensity, enhanced, narrow band (407–420 nm) blue-light source (ClearLight) has been FDA-approved for the treatment of moderate inflammatory acne (122). Red light can also be used to treat acne. It is less effective at photoactivating porphyrins than blue light, but red light can penetrate deeper into the dermis. Red light may also have additional anti-inflammatory properties. Combined blue and red light therapy may be more efficacious than either alone. It can be used twice weekly, taking 15 minutes per session to treat just the face. To treat the face, chest, and back, a 45-minute session is needed. Clinical improvement is maintained for at least one month after the last treatment (121).

Photodynamic Therapy

Photodynamic therapy is another phototherapy option for treating acne. Aminolevulinic acid (ALA) is applied topically one hour prior to exposure to a low-power light source (such as a pulsed excimer laser or a halogen source). The ALA is metabolized in the pilosebaceous units to protoporphyrin IX which is then targeted by the light. This results in the destruction of the sebaceous glands and damage to the hair follicles and epidermis (123,124).

Lasers

Lasers too are beginning to find a role in the treatment of acne. They work by emitting minimally divergent, coherent light that can be focused over a small area of tissue. Pulsed

dye laser (585 nm) can be used at lower fluences to treat acne. Instead of ablating blood vessels and causing purpura, the lower fluence can stimulate procollagen production by heating dermal perivascular tissue (121). The beneficial effects of a single treatment can last 12 weeks (125). The 1450 nm diode laser has also demonstrated significant efficacy in treating acne (126,127). This laser works by causing thermal damage to the sebaceous glands. The concurrent use of a cryogen spray device protects the epidermis (128).

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Topical Botanicals

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INTRODUCTION

Botanicals have been part of cosmetics and toiletries since before recorded history. As early as 10,000 BC scented oils and ointments were used to soften skin and mask body odor (1). The ancient Egyptians freshened their breath by chewing pellets made of ground tamarisk leaves (2) and made perfumes from mixtures of essential oils such as myrrh, chamomile, rose, and cedar combined in vegetable oils of olive, sesame, or almond (1). The Picts of the British Isles made blue body paint from woad (*Isatis tinctoria* L). We know this dye was used as war paint from Roman writings of the time. In ancient Persia, and across the ancient world, henna dyes were used to stain hair and faces and the Egyptians used it to paint their fingernails.

Botanicals aren't just for fragrance and color. Many plant extracts have provided important pharmaceutical drugs. For decades, powdered *Digitalis purpurea* (foxglove) leaf (Powdered Digitalis U.S.P.) has been sold as a prescription drug for congestive heart failure. Tubocurarine, the active constituent from curare arrow poison (derived from the South American vine *Chondrodendron tomentosum*) is used as a skeletal muscle relaxant during surgery. Morphine and codeine (from *Papaver somniferum* L.) are still extremely important analgesics. Many important anti-cancer drugs, such as Paclitaxel (from the Pacific yew tree, *Taxus brevifolia*) and vincristine and vinblastine (both from Madagascar periwinkle, *Catharanthus roseus*, a common garden flower), originally were identified from plants.

Plants have the ability to biosynthesize a stunning array of primary and secondary metabolites. Primary metabolites include those constituents that all plants make and are necessary for plants to function. These include carbohydrates, proteins, lipids, etc. Secondary metabolites are compounds that are not generally found in every species of plant. Flavonoids, polyphenols, terpenoids, and alkaloids are usually classified as secondary metabolites. These compounds perform special functions in the plant such as pollinator attractants, anti-feedants, antimicrobials, and antivirals. Plants don't have the ability to get up and move, so they depend on their biosynthetic abilities to protect themselves and to propagate themselves.

The wide variety of chemical constituents found in plants, many of them highly complex chemical structures, have been used as a biochemical resource by mankind.

Plants have been extensively screened for biological activities that are useful to man, including medical and agricultural uses. The unique and complex structures have often shown new types of biological activity and have been a tool for elucidating aspects of how diseases attack our bodies or how pathogens damage crops. When a new mechanism of action is identified, a synthetic method for producing the compound is often pursued. The compound may be used as a template for the development of new drugs. Synthetic variations of the chemical structure can be analyzed for improved activity, fewer side effects, etc.

Biologically active compounds from plants have the ability to provide real health benefits, and perhaps real cosmetic benefits as well. Today botanicals can be found in everything from foot cream to lipstick. Botanical extracts come in many forms. Some are designed to be easily incorporated into cosmetic formulations, but impart little more than a pretty name to the ingredient deck. Other extracts are prepared in such a way as to optimize any potential benefits that the plant may impart. Some of these extracts are standardized to ensure a known concentration of the active compound and that the concentration of the compound will be consistent from batch to batch.

SELECTING PLANT SPECIES

Selection of potential plant species for cosmetic application should be based on ethnobotanical knowledge or scientific research demonstrating beneficial properties. The safety of the plant and the therapeutic constituent in question also needs to be investigated. Once you have determined what type of benefits or claims you are looking for from a botanical extract and which plants could provide the desired effects, you should investigate if any of the plants in question are threatened or endangered species. The Convention on International Trade in Endangered Species of Fauna and Flora Web site is an excellent resource for this type of information (www.cites.org). If the selected plant has no issues in this area, is it being produced in a fashion that will permit sustained harvesting and is it available in the quantities necessary to support your needs?

Wildcrafting, collecting plants from the wild for commercial uses, has been known to devastate species, or at least a local population of plants, and has led to certain species becoming threatened or endangered. Surprisingly, many plant species used in herbal medicines and extracts are still being collected from the wild (3). Many medicinal plants are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation, and indiscriminate collection. Ornamental species, including native orchids, are under these pressures as well. According to the World Conservation Unit Red List of Threatened Plants, 12.5% (or 34,000 plant species) of vascular plants alone are at risk of extinction (4). Even in North America plants such as ginseng (*Panax quinquefolium*) and goldenseal (*Hydrastis canadensis*) have been notably reduced due to over-harvesting (5). *Limonium wrightii* H., a plant used in traditional Chinese medicine, is no longer found growing wild in Taiwan. In order to meet commercial demands, this plant is now farm grown (3).

SOURCING PLANT MATERIAL

Number of sources and reliability of sources available are important considerations when selecting an extract or a supplier. For instance, lauric acid is used widely in soaps and detergents. It used to be obtained mainly from Philippine coconut oil. However, the price

of coconut oil was highly unstable due to drought, aging plantations, typhoons, pests, and diseases in the Philippines. One good typhoon could wipe out an entire years crop. African oil palm (*Elaeis guineensis*) is also an excellent source of lauric oils. It is grown in Indonesia and Malaysia and other parts of the tropics and is now an important commercial source for lauric acid.

Fu Ling or poria (*Poria cocos*) is a fungus widely used in traditional Chinese medicine. When the SARS outbreak occurred, the demand for poria in Asia was so great that it was virtually impossible for western herb companies to obtain (6). Any plant crop that comes from one specific location could potentially be unavailable or the year's crop wiped out due to weather, natural catastrophe, war, or even epidemic. The number and reliability of sources available should be a consideration when selecting a new botanical.

ACCURATE IDENTIFICATION OF PLANT SPECIES

Historically, plants have been identified by an examination by a plant taxonomist of the leaf, fruit, flower, and other plant parts necessary for proper determination (7). Precise notes regarding the specimen's colleting location, including latitude and longitude, village, county, province or state, and country, and a description and photos of the plants height, width, habit, color, fragrance, etc. in its natural habitat, may also be required. Of course, this type of information is usually not available when purchasing an extract or dried plant material from a vendor.

The U.S. Pharmacopeia (8) has thin-layer chromatography (TLC) methods for identifying certain commonly found dietary supplement herbal products, based upon their chemical constituents. Other analytical techniques such as gas chromatography (GC) or high performance liquid chromatography (HPLC) could just as easily be used.

DNA fingerprinting methods can also be developed for identifying plants (9). This is especially useful to ensure that microbial strains, which seem often to be counted as botanicals in the cosmetic world, have maintained their integrity over multiple generations of serial transfers during the culture maintenance process.

HARVESTING PLANT MATERIAL

There are several things to consider when harvesting plants for extraction. Firstly, the plant material harvested should come from healthy, disease-free plants. The plant material is typically air-dried in arid regions or oven-dried in humid regions (to avoid mold) to a moisture content of $\leq 10\%$. The plant material is then ground or milled to a small particle size, typically 1–10 mm. This provides a larger surface area for extraction and a more exhaustive extraction in a shorter period of time.

For some types of extracts, the fresh plant material is extracted without drying. For example, volatile compounds (e.g., monoterpenes, sesquiterpenes) may evaporate off during the drying process. So, the fresh plant material is often steam distilled or extracted as soon as it is harvested. Certain highly sensitive compounds may be degraded during the drying process by heat, light, oxygen, or even enzymes within the plant material. These may also be extracted shortly after harvest in order to maintain biological activity of the final extract.

It is not uncommon for a desired constituent to be found at varying concentrations in different parts of the plant (Table 1). So, for instance, glaberdines, the skin lightening constituents from licorice, are found in higher concentration in the roots of the licorice

Table 1 Concentration of Constituents in Different Parts of the Plant

Plant	Constituent	Leaf	Root	Other	Reference
Astragalus membra neaceus	Isoflavones	0.55 mg g/l dry wt	3.04 mg g/l dry wt.		(10)
Astragalus membra neaceus	Flavonols	3.54 mg g/l dry wt	0.49 mg g/l dry wt.		(10)
Psychotria brachyceras	Brachycerine	0.1–0.2% dry wt (leaves and green stems)	Not detected in roots	0.3% dry wt. in inflorescences 0.045% dry wt. in mature fruits	(11)
Hydrastis canadensis	5-O-(4′-[β-d-glucopyranosyl]-trans-feruloyl) quinic acid	Not detected	1.0% w/w	2.3% w/w in rhizomes $<0.1\%$ w/w in stems	(12)
Panax quinquefolius	Rg3 ginsenoside	7.5 mg/g	10.6 mg/g		(13)
P. quinque folius	Rg2 ginsenoside	11.3 mg/g	Not detected		(13)

plant. For green tea catechins, the leaf is utilized. The beneficial constituents of Saint John's Wort are highest in the flowers, although a combination of flowers and leaves are often used. The entire aerial, or above ground portion of the plant, may be used in other cases. Other aspects to consider are that constituent concentration can vary depending on the weather, time of year, elevation, soil conditions, fertilizer, age of plant, disease state, etc. (14).

COSMETIC EXTRACTS

Extracts that are designed specifically for cosmetic products come in many forms. They are usually liquid extracts in a cosmetically friendly solvent or solvent blend such as water, butylene glycol, glycerin, vegetable oil, or cosmetic ester. Some of them are standardized to a marker compound, but many are not. Many non-standardized extracts are designed simply to add a botanical name to your ingredient deck, but others have in vitro or clinical data from the vendor indicating various benefits to skin. Sometimes these tests have been carried out by an independent lab and sometimes the vendor's own testing facilities have produced the data. It is important to remember that, unlike academic journal articles, this type of data is not peer-reviewed and outside labs do not often repeat the testing to confirm the results. Some larger cosmetic companies will in fact do their own ingredient testing to confirm vendor claims before they choose to use the ingredient in a personal care product.

In most cases, the goal of producing an extract is to increase the potency of the botanical by concentrating the biologically active constituents. Although it is not uncommon for one particular constituent from the plant to be predominantly responsible for the therapeutic benefit derived from the plant, frequently there will be a series of closely related compounds, or in some cases unrelated compounds, each of which

contributes to some degree to the beneficial properties of the plant. For oral supplements, another benefit to producing an extract is that it reduces the total quantity of plant material that must be ingested to achieve an efficacious dose. In the case of topical applications, as in cosmetics, where the benefit of the digestive process is not available, extraction of the biologically active compound increases the bioavailability of the compound to the skin.

Many types of extraction processes are used commercially to produce extracts. For instance, volatile products, such as essential oils, are often extracted by steam distillation. Lipophillic compounds, such as carotenoids, xanthophylls, and, once again, essential oils or their constituents, are more and more often extracted by CO_2 super critical fluid extraction. This process uses pressure and low heat to convert CO_2 into a fluid phase, or physical state, in which it acts as an excellent non-polar solvent. The polarity can be modified, to some extent, by adding more polar solvents, such as ethanol, to the extraction. A major advantage to this process is that it is "green"; no harmful organic solvents are released into the environment. Enzymes such as cellulases can be used to break down the cell walls of the plant tissue causing the cells to expel their contents into the enzyme solution. Despite the many options, solvent extractions are probably still the most common method for extracting small molecules such as the secondary metabolites of plants.

The general approach for solvent extraction is "like dissolves like." In other words, non-polar solvents will extract non-polar constituents and polar solvents will extract polar constituents. So an extraction process needs to be based on the particular compound, or class of compounds, that provide the beneficial properties of the plant in question.

For a high concentration of the target compound(s) in the final extract, the solvent selection and extraction process development must be based upon optimal extraction and purification of the desired compound. Factors such as solvent choice, extraction duration, temperature, etc. of the extraction process must all be evaluated. While applying heat during the extraction process can reduce the required duration of the process and produce a more exhaustive extraction, some compounds are heat labile. Light and oxygen can also cause degradation of certain compounds. If the target compound is highly labile, reducing the extraction temperature may be required. Nitrogen blanketing may decrease degradation, or the addition of antioxidants can also help to protect some highly labile compounds.

A common extraction process would include macerating, or often refluxing, the dried, milled plant material in an alcoholic solvent or water-alcohol mixture. The benefits of alcohol are that it is a powerful solvent capable of extracting many types of molecules, it can preserve the extract and so eliminate the risk of microbial degradation, and alcohol is relatively volatile and is removed fairly easily once the extraction is complete, by vacuum distillation or evaporation.

Alcohol extracts are generally very complex mixtures of constituents. Due to the fact that alcohol is a powerful solvent, it extracts a wide range of constituents from the plant material. A second purification step is often performed in order to obtain a high level of the biologically active compound in the final extract product. The alcohol may be distilled off and a second purification step applied. A simple and common approach is to perform a second extraction on the dried extract using a different solvent. For example, the alcoholic extract of *Centella asiatica* may be dried down to a paste consistency. The paste is then extracted with acetone and the precipitate is recovered by filtration and dried. The precipitate is assayed and adjusted to 40% saponins, milled and packaged as a commercial extract (15). For other types of extracts, the target compound might be recovered from the filtrate instead of the precipitate. Ideally, a solvent will be found in which the desired constituent is only partially soluble. At elevated temperatures the constituent will dissolve in the solvent and any insoluble material can be filtered out. As the extract is cooled

the solubility of the target compound goes down and, under the right conditions, it crystallizes out of solution. The relatively pure compound is then recovered by filtration

Other commonly used purification techniques include ultrafiltration, nanofiltration, and column chromatography. Ultrafiltration and nanofiltration are excellent purification methods for water-based extracts. These methods use membranes to separate compounds based on molecular size. Column chromatography is a procedure by which solutes are separated by differential migration properties in a system consisting of two or more phases. One phase moves continuously in a given direction and the individual compounds within the phase exhibit different mobilities by reason of differences in adsorption, partition, solubility, molecular size, or ionic charge density. The second phase, a stationary phase, may act through adsorption, as in the case of adsorbents such as activated alumina, silica gel, and ion-exchange resins, or it may act by dissolving the solute thus partitioning it between the stationary and mobile phases.

STANDARDIZATION OF EXTRACTS

An extract that has been purified or formulated to contain a consistent, measurable quantity of a target compound (or sometimes class of compounds) in every batch, is referred to as "standardized." The level of the compound is guaranteed to be within a certain range or above a certain minimum in every batch or lot of the extract that you purchase. The exact level of the compound in a particular batch should be reported on the Certificate of Analysis, which most vendors provide with the extract shipment. Common chemical analytical methods such as HPLC, GC, titration, etc. are generally used to measure the content of the target compound within the extract. Although, in some cases, pharmacological bioassays are used to measure the particular type of biological activity within the extract, for example, enzyme activity.

There are two schools of thought regarding how an extract should be standardized. Some people feel that a virtually pure compound is the most efficacious approach to formulating an effective product. Using one pure plant derived compound eliminates any confounding properties that a more complex extract might have. Botanical extracts can contain extremely complex mixtures of compounds. It is possible that some compounds in the mixture may interfere with or counteract the benefits of the target compound. For example, the polyphenol fraction of St. John's Wort (*Hypericum perforatum*) has been found to have immunostimulating activity, whereas the lipophilic fraction had immunosuppressing properties (16). In a complex mixture, you don't know exactly what you have. Complex extracts may cause difficulties formulating, problems with formula stability, or perhaps occasionally problems with safety such as allergic responses to the final product.

The other school of thought seems to stem mainly from ethnobotanical research. Traditional herbal medicines are usually prepared as teas (aqueous infusions of the fresh or dried plant material). In some cases, different compounds within the tea contribute additional or different therapeutic benefits. For instance, ginger (*Zingiber officinale* R.) contains anti-inflammatory compounds and anti-nausea compounds (17). Using a pure form of one of the anti-inflammatory compounds will not give the full range of benefits derived from the traditional medicine.

Valarian (*Valeriana officinalis*), used as a sedative, is another example of an herbal medicine that appears to contain more than one type of compound responsible for the benefits derived from it (18). The volatile oil contains major constituents, including valerenic acid and its derivatives, which have demonstrated sedative properties in animal

models. Valepotriates and their derivatives, which belong to the iridoid class of molecules, have also demonstrated in vivo sedative activity, but they are very unstable and tend to break down over time, making their activity difficult to assess. Valerian extracts also contain gamma-aminobutyric acid (GABA) and aqueous extracts contain glutamine which may be converted into GABA. These compounds may also contribute to valerians sedative effects. In this case, the herbal medicine contains three different classes of molecules, all of which may to contribute to the sedative benefits of the extract.

Another important consideration is whether the extract is standardized to the correct constituent. As can be seen from the previous examples of herbal medicinals, often the compounds responsible for the therapeutic benefits are not well understood, sometimes not known period. For many years the constituents responsible for the antidepressant benefits of St. John's Wort (*Hypericum perforatum*) were not understood. It was thought that hypericin was the primary active in the herb. Dietary supplements of St. John's Wort were standardized to hypericin, and perhaps still are in some instances. Hypericin is known to be a photosensitizer (19). This property was first recognized in cattle that grazed on this plant. Several instances of photosensitization in people using St. John's Wort herbals have been reported (20–22). Hyperforin is now recognized as the major antidepressant constituent of this plant. Hyperforin has been found to be a strong uptake inhibitor of serotonin, dopamine, noradrenaline, GABA, and L-glutamate (23). Although hyperforin alone is a powerful antidepressant, several other compounds in the plant also appear to contribute to the overall antidepressant benefits from the plant (24). This compound is not a photosensitizer, but it is unstable. Its instability makes it difficult to standardize to and perhaps explains the continued use of hypericin as the marker compound.

QUALITY ISSUES

Quality concerns will differ depending on the particular plant or extract. The typical types of properties that are used to determine the quality or batch to batch consistency of a botanical ingredient would include: appearance, color, odor, botanical characteristics (for plant material), microbial count, pH, residue on evaporation or loss on drying, total ash, acid-insoluble ash, water soluble ash, heavy metal content, alcohol-soluble extractives, water-soluble extractives, foreign organic matter, solvent residue, moisture content, volatile oil content, pesticide residue, and of course the level of marker compound if the extract is standardized. In some cases, a fingerprint method may be developed to ensure that the plant or plant extract is what it claims to be and/or is consistent from batch to batch. This might be a DNA fingerprint, chromatography profile (e.g., TLC, HPLC or GC), or even an IR fingerprint. Generally, five to 10 characteristics are reviewed for a particular product and the assay results will be listed on the Certificate of Analysis which should be available for every batch of product that is purchased.

Microbial contamination is especially common in dried plant material where the microbial counts are generally very high. USP or CTFA plate count methods may be utilized to evaluate this. Irradiation is commonly used to sterilize herbaceous material. Extracts may have microbial issues depending on what solvent was used in the extraction process. Many organic solvents, such as ethanol and methanol, are antiseptics and so will effectively preserve an extract. Other extracts, especially water-based ones, are typically preserved or sterilized.

Pesticide levels can be a concern for some plant based products. The United States Pharmacopeia (8) is a good resource for acceptable levels. A table of 30 or more pesticides

and the maximum limit for each is shown under "General Method for Pesticide Residues Analysis" in the *Chemical Tests* section. Any pesticides not listed are considered unacceptable at any level. These limits were set for dietary supplements, but are a good guideline for cosmetics as well.

Some types of plants have a tendency to accumulate certain heavy metals (14). For instance, mugwort plants (*Artemisia vulgaris* L.) and coneflower roots (*Echinacea* spp.) are known to accumulate iron; black cherry stems (*Prunus serotina* E.) and buckbush stems (*Symphoricarpos orbiculatus* M.) accumulate lead; cassia plants (*Cinnamomum aromaticum* N.) and bladderwrack plants (*Fucus vesiculosus* L.) accumulate mercury (25). Thus, for certain plant materials or extracts heavy metal levels should be assayed and specified on the Certificate of Analysis.

Preservatives, antimicrobials, and/or antioxidants, may be added to extracts and should be identified by the extract manufacturer upon request. Analytical methods such as HPLC may also be applied to identify preservatives within an extract. Ash quantity is often used as a quality specification for extracts. Excessive quantities of ash may indicate the presence of buffers (sometimes used during extraction process to adjust the polarity of the solvent) or drying agents such as silica dioxide.

SAFETY AND TOXICOLOGY

Just because an ingredient is plant-derived doesn't mean it is safe. Just ask Socrates! Poisons such as strychnine come from plants (*Strychnos nux-vomica* L. and other *Strychnos* spp.). And potent allergens, such as the heptadecylcatechols from poison oak (*Toxicodendron diversilobum*) and pentadecylcatedchols from poison ivy (*T. radicans*) are also plant derived (26).

Many plant extracts are considered safe because they are made from ingredients that are Generally Recognized as Safe (GRAS) (27). Toxicology testing is important for other extracts. Highly purified plant constituents, even if they come from plants that are GRAS, may need testing due the increased concentration of the particular constituent. Some ingredients are safe at low levels but cause problems at higher levels. Some of the types of testing that are commonly used to test the safety of cosmetic extracts include repeat insult patch testing (RIPT), cumulative irritation, in vitro mutagenicity (i.e., Ames test), in vitro cell culture methods for assessing potential ocular and/or dermal irritation (e.g., Bovine ocular assay, Irritection™, Eyetex™, Skintex™, etc.), and photosensitization. This may not be adequate in all cases.

A noteworthy example is sanguinarine, an alkaloid extracted from Bloodroot (*Sanguinaria canadendid*). Viadent used sanguinarine as an antiplaque ingredient in their toothpaste and mouthwash. Despite significant toxicology and clinical testing demonstrating the safety of this compound (28–37), a study was conducted by researchers at Ohio State University (38), which showed a strong correlation between the development of oral leukoplakia (potentially cancerous mouth lesions) and the use of oral products containing sanguinarine. Several follow-up studies (39–42) have confirmed this correlation. Surprisingly, other studies appear to indicate that sanguinarine may actually have potential as an anti-cancer agent (43–45). This demonstrates how complicated and confusing these safety and toxicology issues can be. Long-term safety can only be demonstrated by long-term use. When Colgate-Palmolive purchased the brand, they removed the sanguinarine.

CONCLUSIONS

Plant-derived compounds have the ability to deliver real benefits. Considerations in choosing a plant product should include an investigation of the status of the species (is it endangered or threatened), whether sustainable harvesting practices are being used, and whether there is a reliable source with the quantities needed. Many extraction and purification methods are used commercially depending on the desired qualities of the end product. As with all cosmetic ingredients, standard quality assurance techniques should be followed to ensure the quality and consistency of the product or extract. The safety and toxicology of the material should be investigated to eliminate or reduce the risk of harm to consumers.

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