Chapter 13

# Retinoid Therapy for Photoaging

13

Laure Rittié, Gary J. Fisher, John J. Voorhees

Contents



## 13.1 Introduction

Since the initial report that described that topical all-*trans*-retinoic acid (RA) had a beneficial effect on photoaging [1], the effectiveness of topical all-*trans*-RA in the treatment of photodamaged skin has been demonstrated in a large number of controlled clinical studies [2–7]. Discoveries during the past 10 years have provided detailed knowledge regarding the fundamental molecular mechanisms of action of all-*trans*-RA in mammalian tissues. In spite of this knowledge, large gaps exist in our understanding of the molecular basis by which all-*trans*-RA improves the appearance of photodamaged skin.

The term photoaging relates to alterations that occur in skin that is chronically exposed to sunlight. In sun-exposed skin, photoaging is superimposed on natural, intrinsic aging, which occurs in all skin. Subject-matched clinical, histological, and biochemical analysis of sun-exposed and sun-protected skin readily reveals that sun exposure undermines the appearance and function of human skin to a much greater degree than natural aging. Photoaged skin appears dry, wrinkled, lax and unevenly pigmented, and has brown spots [8]. Histologically, photoaged skin shows variable epidermal thickness, large and irregular grouped melanocytes, lack of compaction of the dermis, elastosis, and mild inflammatory infiltrate. Photoaging is a cumulative process, and as such, its severity is highly correlated with age. Conversely, photoaging is inversely correlated with skin pigmentation, since darker skin provides a more effective barrier against penetration of sunlight. During the past 20 years, great progress has been made in our understanding of the molecular mechanisms responsible for photoaging.

In this chapter, we first describe the molecular mechanisms by which ultraviolet (UV) irradiation from the sun damages human skin, focusing on damage to collagens, which are the major structural components of skin connective tissue. We do not describe in detail UV damage to DNA, which is addressed in chapter 4. We present evidence that topically applied retinoids can both prevent UV-induced skin damage and repair photodamaged skin. In addition, we describe our current understanding of the mechanism of action of retinoids. Finally, we review some of the side effects associated with retinoid therapy.

## 13.2 UV-Mediated Damage to Skin Connective Tissue

UV irradiation from the sun causes a large number of deleterious effects to human skin, including sunburn, immunosuppression, and skin cancer, in addition to premature aging. Solar UV radiation that reaches the surface of the earth is subdivided by wavelength into UVB (290–320 nm), UVA2 (320–340 nm) and UVA1 (340–400 nm). The energy inherent in UV radiation can be absorbed by many different cellular components within the skin, and consequently elicits a variety of cellular responses brought about by coordinated activation of signal transduction pathways that result in alterations in gene expression. A common initiating event following UV irradiation is the photochemical generation of reactive oxygen species (ROS), which can modify different cellular components (i.e. DNA, proteins, lipids), and cause oxidative stress. In this section, we review the mechanisms by which UV light activates specific signaling pathways, and thereby modifies gene transcription and damages human skin connective tissue.



### 13.2.1 UV Irradiation Induces Activation of Signaling Pathways

The primary mechanism by which UV radiation initiates molecular responses in human skin is via photochemical generation of ROS, including superoxide anion ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radical ($HO^\cdot$), and singlet oxygen ($^1O_2$) [9]. ROS are produced by energy and/or electron transfer from UV-absorbing chromophores in the skin. Many chromophores have been proposed as endogenous ROS generators, including tryptophan [10], nicotinamide adenine dinucleotide phosphate (NADPH), riboflavin, porphyrin [11], and *trans*-urocanic acid [12]. ROS may also be generated by UV irradiation of dermal collagen and elastin [13], and soluble proteins modified by advanced glycation end products (AGE), such as albumin [14, 15]. Increased ROS production is detected within one minute after UVA irradiation of mouse skin in vivo, and can be prevented by topical application of superoxide dismutase [16].

In human skin, UV-induced liberation of ROS is responsible for stimulation of numerous signal transduction pathways via activation of cell surface cytokine and growth factor receptors. These include epidermal growth factor receptor (EGF-R) [17, 18], insulin receptor [19], platelet derived growth factor receptor (PDGF-R) [20], tumor necrosis factor-α receptor (TNF-α-R) and interleukin-1 receptor (IL-1R) [21]. UV irradiation of skin cells induces a substantial response due to simultaneous activation of multiple receptors [21]. EGF-R activation occurs within 15 minutes following UV exposure (twice the minimal erythema dose or MED) of human skin in vivo and remains elevated for two to four hours [22].

UV-mediated activation of EGF-R is ligand-independent, since the ligand-binding domain is not required for UV activation of the receptor [20]. However, direct activation of EGF-R by UV light has never been demonstrated. Alternatively, it has been suggested that UV-induced growth factor and cytokine receptor activation is mediated by ROS-mediated inactivation of protein tyrosine phosphatases (PTPs) [20, 23]. PTPs act on tyrosine kinase receptors to maintain a low basal phosphorylation state of the receptor. PTPs act in concert with tyrosine kinases to maintain a dynamic equilibrium between phosphorylation and dephosphorylation of receptors. ROS formed after UV irradiation inactivate PTPs via reversible oxidation of a

critical cysteine residue within the catalytic site, common to all PTPs [24, 25]. Thus, UV irradiation of cells can increase the half-life of EGF-R phosphorylated tyrosine(s) [20].

Recently, it has been shown that in addition to reversible oxidative inactivation of PTPs, UVA and UVB can trigger degradation of certain PTPs in cultured cells. This degradation is mediated by the protease calpain, and requires both activation of calpain and the oxidative alteration of PTP by UV irradiation [26]. The PTPs involved in responses of human skin to UV irradiation remain to be identified.

Downstream signaling induced by UV-mediated activation of cell surface receptors is similar to that which follows ligand binding. Autophosphorylation of cell surface receptors results in recruitment of adaptor and regulatory proteins that mediate downstream signaling, including activation of the three families of mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinase (ERK), c-Jun amino-terminal kinase (JNK), and p38. Activation of protein kinase-mediated signaling pathways occurs within one hour after UV irradiation of human skin in vivo, and is maximal four hours after UV irradiation. Activation of all three MAP kinase modules occurs throughout the epidermis and upper dermis [27]. Activated JNK and p38 catalyze the functional activation of transcription factors c-Jun, and activating transcription factor-2 (ATF-2), respectively. Activated ATF-2 binds c-Jun promoter and upregulates c-Jun protein transcription [28]. C-Jun partners with c-Fos, which is constitutively expressed in human skin and is not further elevated after UV irradiation [22], to form activator protein (AP)-1 transcription factor complex. Stimulation of AP-1 by UV irradiation induces gene expression of AP-1-regulated matrix metalloproteinases (MMPs), i.e. MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1), and MMP-9 (gelatinase B) [29]. In parallel, AP-1 negatively regulates type I procollagen synthesis by dermal fibroblasts [30]. MMP induction occurs throughout the epidermis and upper dermis. Induction of MMP-1, -3, and -9 mRNA is detected within eight hours following UV irradiation of human skin in vivo, and is followed by increased protein synthesis, and enzyme activities [29, 31]. MMP levels remain elevated for two to three days after a single acute UV exposure in human skin in vivo [29]. Increased levels of endogenous glutathione induced by topical application of N-acetyl cysteine (NAC) reduce UV-induced activation of c-Jun and consequent induction of MMPs [32]. This observation is consistent with the involvement of ROS in UV-mediated activation of MMPs.

UV irradiation of skin also upregulates nuclear factor-κB (NF-κB). The NF-κB pathway regulates expression of genes primarily involved in immune and inflammatory responses. In the absence of stimuli, NF-κB is sequestered in the cytoplasm, bound to its inhibitor IκB. Cytokine activation of NF-κB is mediated by stimulation of IκB kinase (IKK), which phosphorylates IκB. Phosphorylated IκB is rapidly degraded and releases NF-κB, which translocates into the nucleus where it activates target genes. However, this mechanism is not applicable to UV-induced NF-κB activation since no IKK activation is detected after UV irradiation [33, 34]. As opposed to TNF-α, UV irradiation activates NF-κB in a delayed (three to six hours after irradiation) and relatively weak manner [33, 34]. Recently, it has been demonstrated that UV irradiation downregulates IκB through inhibition of IκB synthesis [35]. In the absence of ongoing IκB synthesis, the cellular IκB content is slowly depleted by natural degradation, eventually leading to NF-κB activation. It has also been demonstrated that the carboxyterminal zinc finger domain of NEMO (regulatory subunit of IKK) is required for the UV-induced NF-κB signaling pathway [36]. Recently, it has been reported that the protein kinase MAPK-activated kinase-2, which is activated by p38, phosphorylates heat shock protein 27 (Hsp27), which in turn binds to IKKβ (one of the two catalytic subunits of IKK). Binding of Hsp27 to IKKβ inhibits IKK activity [37]. UV irradiation strongly induces p38 in human skin in vivo [28]. Therefore, it is possible that UV-induced p38 activation weakens NK-κB activation and downregulates IKK activity to below the level of detection.

Once translocated into the nucleus, NF-κB upregulates the synthesis of numerous cytokines including IL-1β, TNF-α, IL-6, and IL-8 [38]. These cytokines are secreted, and act in an

autocrine and/or paracrine manner by binding to their cell surface receptors on epidermal and dermal cells, thereby activating AP-1 and NF-κB and amplifying the UV response.

### 13.2.2 UV Irradiation Induces Degradation of Skin Collagen

UV-induced activation of MMPs results in excessive degradation of extracellular matrix (ECM) components in the dermis. ECM is the substrate for cell adhesion, growth, migration, and differentiation, and provides mechanical support for tissues and organs. ECM proteins are secreted by fibroblasts in the dermis, and feedback mechanisms allow the fibroblasts to adapt to alterations in the ECM. This bidirectional interaction between ECM and ECM-supporting fibroblasts is critical for optimal function of skin connective tissue.

Type I collagen is the most abundant structural protein in skin connective tissue. It accounts for about 85% of total dermal protein. Type III collagen interacts with type I collagen, and is present at approximately one-tenth the level of type I collagen. Type I and III collagens are composed of three polypeptide chains, organized in a rod-like triple helical structure. Type I and type III collagens are synthesized by fibroblasts in the dermis, as precursor proteins (procollagens) containing globular domains at each of their ends. These globular portions, referred to as N-terminal and C-terminal propeptides, allow procollagens to be secreted from the cell as a soluble monomeric protein. Once secreted, the propeptides are enzymatically removed by specific proteases. Cleavage of the C-terminal propeptide precedes cleavage of the N-terminal propeptide. Removal of the propeptides allows the collagen molecules to spontaneously assemble into fibers. Newly formed collagen molecules assemble into existing collagen fibers, and undergo intermolecular crosslinking to form mature collagen fibers.

Collagen crosslinking is catalyzed by the enzyme lysyl oxidase [39]. Collagen fibers are arranged in orderly arrays in association with other ECM proteins to form collagen bundles. Collagen bundles provide strength and resiliency to the skin, and are very resistant to enzymatic degradation. The half-life of skin collagen is estimated to be several years. Degradation of collagen fibers requires the action of collagenases. Three mammalian collagenases exist, MMP-1, MMP-8, and MMP-13, which have the unique capacity to initiate cleavage of mature collagen. As described above, UV irradiation induces MMP-1. MMP-8 is primarily expressed by neutrophils, which infiltrate skin in response to immune stimuli induced by UV irradiation. Although MMP-8 levels increase in human skin following UV irradiation as a consequence of neutrophil influx, MMP-8 remains largely in an inactive state, and therefore does not significantly contribute to the collagen degradation observed following UV exposure [27]. MMP-13 is not induced by UV irradiation in human skin in vivo. Therefore, MMP-1 is the primary collagenase that is responsible for initiation of collagen breakdown in UV-irradiated human skin. Once cleaved by a collagenase, fragmented collagen can be further degraded by elevated levels of MMP-3 and MMP-9 [40].

### 13.2.3 UV Irradiation Inhibits Production of New Collagen

In addition to causing degradation of mature collagen, acute UV irradiation transiently inhibits new synthesis of type I and type III procollagens [30]. Following UV irradiation of human skin in vivo, type I procollagen protein is significantly decreased within eight hours, is maximally reduced by approximately 70% by 24 hours, and returns to baseline levels within two to three days [30]. Loss of procollagen production is localized to the upper one-third of the dermis, reflecting the penetration of UV irradiation into the skin. At least two mechanisms contribute to downregulation of procollagen synthesis. First, as mentioned above, UV irradiation of human skin activates the transcription factor AP-1, which negatively regulates transcription of both genes that encode type I collagen chains, COL1A1 and COL1A2 [41, 42]. Second, UV irradiation impairs the signaling of

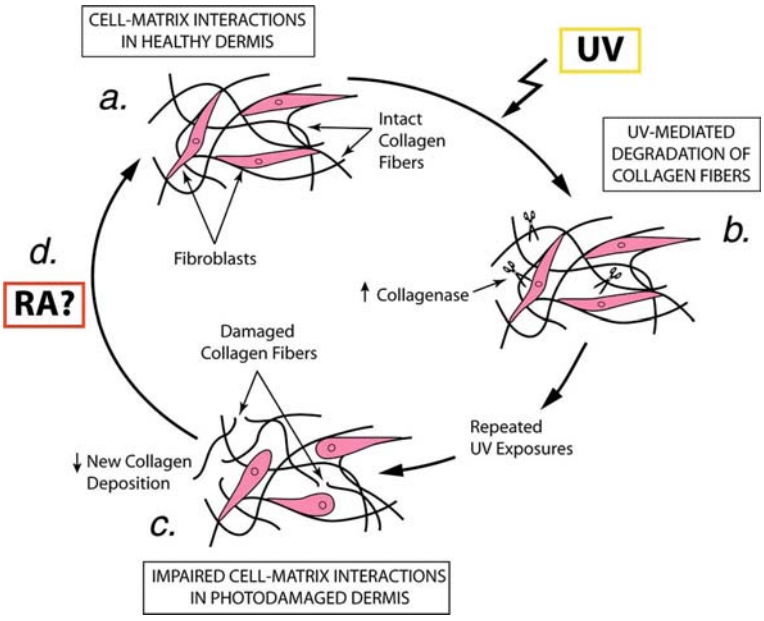


**Fig.13.1.** Mechanism for UV-mediated collagen depletion in human skin. In vivo and in healthy dermis, fibroblasts interact with intact collagen fibers at numerous adhesion points (*a*). UV irradiation increases collagenase, which degrades collagen fibers (*b*). With repeated UV exposures, damaged collagen fibers accumulate, thus impairing cell-matrix interactions (*c*). Impaired cell-matrix interactions are responsible for sustained decrease in collagen production in photodamaged skin. RA-mediated restoration of collagen formation (*d*) is the main underlying process in the repair of photodamaged skin

TGF-β, the major profibrotic cytokine, downregulating the type II TGF-β receptor and, to a lesser extent, increasing expression of the inhibitory regulator of the TGF-β signaling pathway, Smad7 [43, 44].

### 13.2.4 Cumulative Collagen Damage Contributes to the Phenotype of Photodamaged Skin

Photodamaged human skin is characterized by sustained downregulation of type I procollagen production, compared with matched individual sun-protected skin [45, 46]. Procollagen downregulation correlates with the clinical severity of photodamage [46], and therefore appears to reflect a substantial degree of accumulated damage.

Interestingly, the inherent capacity of fibroblasts in severely photodamaged human skin to produce procollagen is not impaired. When grown in vitro, fibroblasts cultured from severely photodamaged skin and those cultured from sun-protected skin synthesize equal amounts of procollagen [47]. As described above, acute UV irradiation of skin induces transient activation of MMPs, with subsequent degradation of mature collagen. With repeated sun exposures over many years, fragmented collagen accumulates in photodamaged skin [47, 48]. In vitro treatment of collagen gels with MMP-1 generates collagen fragments that are similar to those observed in photodamaged human skin in vivo [48]. When dermal fibroblasts are seeded in a collagen gel pretreated with MMP-1, procollagen production is dramatically inhibited, compared to that from fibroblasts cultured on intact collagen gels [49]. Interestingly, removal of partially degraded collagen by treatment of the gels with MMP-9 restores procollagen biosynthesis. This loss of procollagen production results, at least in part, from reduced mechanical tension between the

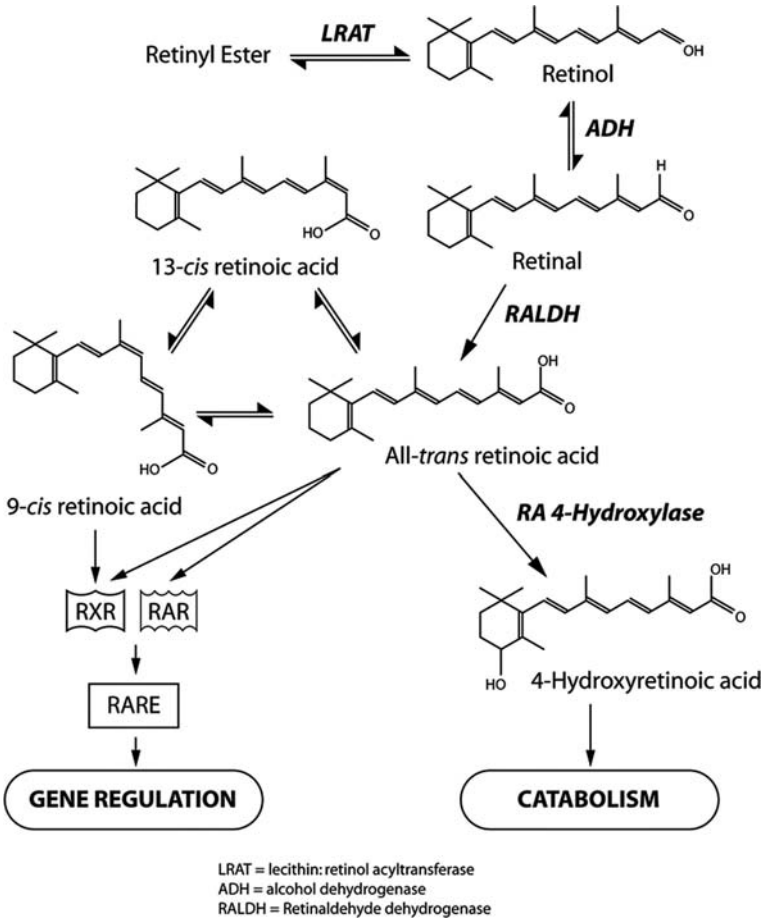


**Fig. 13.2.** Retinoid metabolism in the target cell



fibroblasts and the partially degraded collagen gel.

Fibroblasts bind to collagen through specific receptors, integrins, which they display on their cell surface. Integrin binding allows fibroblasts to exert contractile forces on the collagen matrix, and in turn experience mechanical resistive forces exerted by the collagen matrix. Such mechanical tension has been demonstrated in model systems to influence a wide range of cellular behavior, including procollagen production by skin fibroblasts [50]. Therefore, it has been proposed [51] that accumulation of fragmented collagen in photodamaged skin weakens the resistive forces of the ECM, thereby reducing mechanical tension on fibroblasts, which in response downregulate procollagen synthesis (Fig. 13.1). This scenario describes a continual, self-sustaining negative feedback pathway for loss of skin collagen, since UV exposure generates fragmented collagen by MMP-mediated degradation, and fragmented collagen inhibits procollagen production. In order to break this downward spiral, one must introduce a therapeutic agent that stimulates procollagen production in photodamaged skin. All-*trans*-RA has been demonstrated to be such an agent.

## 13.3 Retinoic Acid Metabolism in Skin

Retinoids constitute a group of natural and synthetic compounds characterized by vitamin A-like biological activity. Vitamin A (all-*trans*-retinol, ROL) and other natural retinoids,

including all-*trans*-RA, play a critical role in embryogenesis, reproduction, vision, regulation of immunity, and epithelial cell differentiation. Retinoids are obtained from the diet, absorbed by the intestine, stored in the liver as retinyl esters (REs), and transported in the circulation to target cells as ROL. The mobilization of ROL from liver cells is regulated by the plasma concentration of unbound retinol binding protein (RBP). When ROL is released into the circulation, it binds RBP for delivery to target tissues. Within the target cell, ROL can be stored at the plasma membrane as REs, or converted into RA to exert biological effects. Conversion of ROL to RA consists of a two-step reaction (Fig. 13.2). First, ROL is converted into retinaldehyde (retinal, RAL). This rate-limiting step in RA metabolism is catalyzed by the enzyme alcohol dehydrogenase (ADH) [52]. The second step is the conversion of RAL into RA. This reaction, catalyzed by retinaldehyde dehydrogenase (RALDH), is irreversible. This is the reason why administration of RA to vitamin A-deficient animals does not increase RAL or ROL levels. In the cell, RA is not stored and its concentration is tightly controlled.

RA is bound within the cell cytoplasm by cellular RA binding protein (CRABP). RA bound to CRABP can undergo one of two fates: delivery to nuclear RA receptors (RARs), or catabolism into inactive forms. Catabolism is catalyzed by RA-4-hydroxylase, which belongs to the cytochrome P450 family [53]. RA concentrations in human skin are tightly regulated: topical application of RA to human skin in vivo increases RA degradation via induction of RA-hydroxylase [54], and decreases RA biosynthesis by increasing ROL-esterifying activity [55].

RA exerts its biological effects through binding to RARs, which belong to the superfamily of nuclear steroid hormone receptors. RARs partner with retinoid X receptors (RXR) to form functional ligand-activated transcription factors. RAR and RXR are each encoded by three separate genes, RAR-α, -β and -γ and RXR-α, -β and -γ. Among the different isoforms, adult human skin expresses predominantly RAR-γ and RXR-α [56, 57]. RA exists in three different stereoisomeric conformations: all-*trans*, 9-*cis*, and 13-*cis*. RARs bind all-*trans*-RA and 9-*cis*-RA; RXRs exclusively bind 9-*cis*-RA; neither RARs nor RXRs bind 13-*cis*-RA [58]. Upon binding of RA to RAR, RAR/RXR heterodimers form functional multimeric protein complexes that regulate transcription of target genes. Target genes contain specific DNA sequences in their promoter regions called RA response elements (RAREs) to which the RAR/RXR complexes bind [59].

## 13.4 Topical Retinoids Prevent Photoaging

Studies have shown that topical all-*trans*-RA can prevent UV-induced skin responses that lead to degradation and down-regulation of type I collagen in human skin in vivo. Pretreatment of human skin with RA inhibits UV-mediated induction of c-Jun protein and activation of AP-1 [28]. Inhibition of AP-1 by RA seems to occur through a mechanism distinct from transrepression [60, 61]. RA inhibits UV induction of c-Jun protein, but not c-Jun mRNA [28]. Whether RA-mediated inhibition of c-Jun protein induction results from decreased c-Jun protein synthesis and/or increased c-Jun protein degradation is not known.

RA inhibition of AP-1 activity results in reduction of UV-mediated induction of MMP-1, MMP-3, and MMP-9 mRNA and proteins [29], thereby preventing generation of fragmented collagen after UV exposure.

## 13.5 Topical Retinoids Repair Photoaging

### 13.5.1 Use of All-*Trans*-Retinoic Acid in the Treatment of Photoaging

In a pilot study, Kligman *et al.* found that all-*trans*-RA cream (tretinoin, Retin-A), which is used for the treatment of acne vulgaris, could partially reverse structural skin damage associated with photoaging [1]. In this vehicle-controlled, albeit unblinded study, the authors showed that 6 to 12 months of daily treatment

with 0.05% tretinoin applied to the face and forearms induced a thickening of the atrophic epidermis, elimination of dysplasia and atypia, promoted a more uniform dispersion of melanin and neoformation of collagen and blood vessels. These observations were confirmed by several vehicle-controlled double-blind studies, the first of which was reported in 1988 by Weiss *et al.* This study demonstrated that 0.1% tretinoin cream used once daily for 4 months causes significant clinical improvement primarily in fine facial wrinkles, sallowness, looseness of the skin, and hyperpigmented macules (also called "liver spots" or actinic lentigines) [2]. Numerous subsequent clinical studies have confirmed these initial observations using 0.05% tretinoin for 3 to 6 months [3–6]. Interestingly, 0.1% and 0.025% tretinoin produce similar clinical and histological improvement of photoaging [7]. At a concentration of 0.02%, tretinoin is still effective for the treatment of photoaging [62], but lower doses (i.e. 0.01% and 0.005%) have no significant advantages over placebo [6]. When treatment was extended to a total of 9 months or more, persistence of clinical improvement in fine wrinkles and skin roughness has been reported [7, 63], even despite a reduction of dose or frequency of application after the first 4 months [64].

Higher doses of tretinoin are also effective in treating photoaging. In a double-blind placebo-controlled study, Rafal *et al.* found that 83% of patients treated with 0.1% tretinoin daily for 10 months, in contrast to 23% of placebo-treated patients, showed lightening of actinic lentigines. These results were confirmed by histology which showed a significant decrease in epidermal pigmentation in biopsy specimens. The improvement was sustained in subjects examined 6 months after the end of treatment [65]. This same treatment regimen also increased collagen skin content compared to vehicle-treated individuals [45].

In one study, long-term treatment was accompanied by loss of epidermal hyperplasia, suggesting that epidermal thickening is not directly related to clinical improvement [63]. In contrast, retinoid-mediated improvement of photoaging is associated with increased collagen I formation [45], reorganization of packed collagen fibers [66], and increased numbers of type VII anchoring fibrils [67]. Solar elastosis is not substantially reduced; however, it tends to reside lower in the dermis, presumably due to accumulation of new collagen in the upper dermis, below the dermoepidermal junction.

Two observations regarding retinoid therapy of photoaged skin are very consistent. First, the magnitude of effects is largest in skin that originally shows the greatest degree of photodamage. Second, up to 92% of subjects using tretinoin in various studies report erythema and scaling at the site of application. This major side effect is referred to as "retinoid dermatitis", and can be a limitation to the use of retinoids for treatment of photoaging (see below).

### 13.5.2 Use of Other Retinoids in the Treatment of Photoaging

Because of the significant side effects associated with tretinoin therapy, the effectiveness of several other retinoids in the treatment of photoaged skin has been studied, ideally without causing irritation. For instance, 0.1% isotretinoin (13-*cis*-RA) cream used once daily for 8 months results in a reduction in wrinkles and actinic lentigines, accompanied by an increase in epidermal thickness [68]. However, significant erythema has been reported in 65% of subjects treated on the face with isotretinoin in contrast to 25% of subjects treated with vehicle. A statistically significant improvement of overall appearance of photodamaged skin has also been reported after 3 months of treatment with 0.05% isotretinoin followed by 0.1% for the next 6 months [69, 70].

Topical all-*trans*-RAL has been shown to increase epidermal thickness, increase differentiation markers and upregulate CRABP-II when used at 0.5%, 0.1% and 0.05% for 1 to 3 months in vivo [71]. RAL significantly reduces wrinkles, albeit to a lesser extent than tretinoin [72]. RAL also appears to be less irritating (23% of treated subjects) than tretinoin (32%).

ROL mimics the activity of 0.025% RA with respect to epidermal thickening when applied topically at 1.6% to human skin in vivo [73]. Like RA, ROL enhances expression of CRABP-

II, but is much less irritating than RA [73]. ROL and RAL are converted to RA by human keratinocytes, and this conversion is required for biological activity [74].

### 13.5.3 Retinoid Mechanism of Action

Despite extensive evidence demonstrating beneficial effects of topical retinoids in preventing and treating the clinical aspects of photodamaged skin, the detailed molecular basis of this activity remains elusive. Initially, irritation and scaling were thought to be the mechanism underlying retinoid-induced repair. However, several lines of evidence have dispelled this notion. First, two concentrations of tretinoin, 0.025% and 0.1%, were compared for their efficacy and irritancy [7]. Used once daily for 11 months, the two concentrations of tretinoin improved photoaged skin to similar extents, but 0.1% tretinoin was significantly more irritating. Second, topical retinyl palmitate (0.15%) induces skin irritation without demonstrating any advantages over placebo in treating photoaging [75]. In addition, non-retinoid agents that induce irritation and scaling do not enhance collagen synthesis in mouse skin [76]. These results demonstrate that irritation can be separated from efficacy, although irritation is an inherent side effect of retinoid therapy (see below).

The ability of retinoids to restore collagen formation is thought to be the main underlying mechanism by which the appearance of photodamaged skin is improved [45]. RA increases TGF-β in mouse skin [77, 78]. Since TGF-β is a major fibrotic cytokine, its induction by RA may underlie the ability of RA to induce collagen synthesis. However, the role of TGF-β in repair of photodamaged human skin remains to be investigated.

Studies of retinoid-mediated collagen induction in photodamaged human skin have been hampered by the lack of a suitable in vitro model. In vitro cultures of dermal fibroblasts constitutively produce high levels of collagen, which cannot be substantially increased by RA. As mentioned above, culturing fibroblasts in a collagen gel pretreated with human MMP-1 results in downregulation of collagen, as is observed in photodamaged skin [49]. Whether RA can restore collagen formation in this model remains to be determined.

Dermal ECM is a complex matrix composed of many structural components besides type I and type III collagens. Many ECM components have been reported to be altered in photodamaged skin, including elastin [79], anchoring fibrils, proteoglycans, and glycosaminoglycans [80]. It is possible that the mechanisms by which retinoids improve dermal ECM involves action on several dermal components in addition to collagen. For instance, RA increases fibrillin I, a component of microfibrils that are associated with elastic fibers [81]. RA also increases hyaluronic acid content in porcine skin [82] and in human skin organ culture [83]. Finally, it should be noted that, by stimulating collagen formation, RA would be expected to improve interactions between fibroblasts and ECM, which are impaired in photoaged dermis (Fig. 13.1).

## 13.6 Side Effects of Retinoids

Up to 92% of subjects who used tretinoin in various clinical studies have reported "retinoid dermatitis", i.e. erythema and scaling at the site of application [2]. The condition usually peaks early in therapy and disappears when treatment is discontinued. Scaling is the major deterrent to topical retinoid therapy and is often a limitation to the use of retinoids. Recent progress has been made in understanding the mechanisms by which retinoids induce epidermal hyperplasia.

Irritation may be explained by an overload of RA concentration in the epidermis. Epidermis is a non-homogeneous tissue comprising undifferentiated keratinocytes (basal layer) and differentiated cells (suprabasal layers). In normal human skin, the capacity of undifferentiated basal keratinocytes to esterify ROL is four times greater than that of differentiated suprabasal keratinocytes [55], and RA binding capacities increase with keratinocyte differentiation [84]. This scheme is consistent with the observation that a gradient of free RA influences keratinocyte differentiation and proliferation, with the concentration decreasing from the basal to the superficial layers [85]. When human skin

is treated with topical RA, the skin concentration of RA increases enough to activate gene transcription over retinoid receptors [86]. Exogenous RA enhances proliferation of basal keratinocytes [87], which induces an accelerated turnover of epidermal cells, and thickening of the epidermis [2, 88]. Retinoid treatment of normal human skin in vivo also decreases the cohesiveness of the stratum corneum, impairing the skin barrier and leading to an excessive scale.

RA-induced skin hyperplasia requires functional retinoid receptor [89, 90], and is primarily mediated by RARs [90–92]. The intensity of irritation and scaling is directly related to the amount of RA administered to the skin, since lowering the concentration of topical RA reduces irritation, but does not impair biological activity [7].

The involvement of EGF-R (ErbB1) in retinoid-mediated epidermal hyperplasia has been demonstrated in several in vivo and in vitro studies. RA-induced epidermal hyperplasia is triggered by increased secretion of two EGF-R ligands, heparin-binding epidermal growth factor (HB-EGF) and amphiregulin, in suprabasal cells of normal epidermis in vivo (Rittié *et al.*, in preparation). Blockage of HB-EGF or amphiregulin with antibodies (Rittié *et al.*, in preparation) or inhibition of EGF-R phosphorylation by specific inhibitor [87] strongly reduces the RA-induced epidermal hyperplasia in human skin organ cultures. In vivo, topical application of genistein, an inhibitor of tyrosine kinase compatible with topical use, blocks retinoid-induced hyperproliferation of epidermis in human skin (Rittié *et al.*, in preparation). Interestingly, inhibition of retinoid-associated hyperplasia does not impair the ability of retinoid to increase collagen synthesis by fibroblasts in skin organ culture [87]. It would be interesting to determine whether the separation of these two features can be achieved in vivo for the treatment of photoaging.

Topical application of RA precursors causes less irritation than application of RA itself. These precursors do not bind retinoid receptors and must be converted within the cell into RA to exert biological activity [74]. Interestingly, the extent of irritation is inversely proportional to the number of steps required for conversion of the precursor into RA. For instance, 13-*cis*-RA is less irritating than all-*trans*-RA [68], probably because it needs to be isomerized into 9-*cis*-RA or all-*trans*-RA in order to bind retinoid receptors [58]. Moreover, RA has been shown to be more irritating than RAL [72], which is more irritating than ROL [93], the conversion of RAL and ROL into RA being a one- and two-step process, respectively (Fig. 13.2). Failure to detect significant accumulation of RA in ROL-treated skin [73] shows that RA synthesis is tightly regulated, and RA is rapidly used and degraded. Overloading of RA in skin cells is likely to induce skin irritation.

Concern has been voiced that repeated use of topical tretinoin might increase RA plasma concentrations. This issue was carefully addressed and rejected in different studies showing that topical tretinoin treatment has no effect on endogenous plasma levels of RA or its metabolites [68, 94]. Percutaneously absorbed tretinoin represents approximately 1–2% of a single topical dose applied daily for up to one year [94]. Therefore, 1 ml of 0.05% (500 ng/ml) tretinoin applied topically would provide a maximum of 10 ng RA in the bloodstream (retinoid plasma concentration of 1–4 µg/l). Another study has shown showed that daily application of 0.1% tretinoin for 8 months to the face, forearms and hands does not significantly increase plasma retinoid levels [68]. This is certainly the reason why topical tretinoin is not associated with a detectable risk of major congenital disorders [95], as opposed to oral retinoids that are teratogenic when administered during early pregnancy [96].

## 13.7 Conclusions

Photoaging is caused by the skin's reaction to chronic sun exposure. Topical retinoids are effective in treating clinical signs of photoaging, including fine wrinkles, coarseness and laxity. Retinoid dermatitis is inherent to retinoid therapy, since it is mediated by nuclear retinoid receptors. Irritation can be minimized by reducing dose and frequency of treatment. Understanding the molecular mechanisms of action of retinoids in photoaging will provide pharmacological targets for the development of non-reti-

noid compounds that have the beneficial properties of retinoids, without the unwanted side effects.

## Acknowledgements

During the writing of this chapter, Laure Rittié was supported by a grant from Pfizer, Inc.

## References

1. Kligman A, Grove G, Hirose R, Leyden J (1986) Topical tretinoin for photoaged skin. J Am Acad Dermatol 15:836–859
2. Weiss J, Ellis C, Headington J, Tincoff T, Hamilton T, Voorhees J (1988) Topical tretinoin improves photoaged skin. JAMA 259:527–532
3. Leyden J, Grove G, Grove M, Thorne E, Lufrano L (1989) Treatment of photodamaged facial skin with topical tretinoin. J Am Acad Dermatol 21:638–644
4. Lever L, Kumar P, Marks R (1990) Topical retinoic acid for treatment of solar damage. Br J Dermatol 122:91–98
5. Weinstein G, Nigra T, Pochi P, Savin R, Allan A, Benik K, et al (1991) Topical tretinoin for treatment of photodamaged skin. A multicenter study. Arch Dermatol 127:659–665
6. Olsen E, Katz H, Levine N, Shupack J, Billys M, Prawer S, et al (1992) Tretinoin emollient cream: a new therapy for photodamaged skin. J Am Acad Dermatol 26:215–224
7. Griffiths C, Kang S, Ellis C, Kim K, Finkel L, Ortiz-Ferrer L, et al (1995) Two concentrations of topical tretinoin (retinoic acid) cause similar improvement of photoaging but different degrees of irritation: a double-blind, vehicle-controlled comparison of 0.1% and 0.025% tretinoin creams. Arch Dermatol 131:1037–1044
8. Lavker R (1995) Cutaneous aging: chronologic versus photoaging. In: Gilchrest B (ed) Photodamage, vol 1. Blackwell, Cambridge, pp 123–135
9. Hanson K, Clegg R (2002) Observation and quantification of ultraviolet-induced reactive oxygen species in ex vivo human skin. Photochem Photobiol 76:57–63
10. McCormick J, Fischer J, Pachlatko J (1976) Characterization of a cell-lethal product from the photooxidation of tryptophan: hydrogen peroxide. Science 191:468–469
11. Dalle Carbonare M, Pathak M (1992) Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B 14:105–124
12. Hanson K, Simon J (1998) Epidermal trans-urocanic acid and the UV-A-induced photoaging of the skin. Proc Natl Acad Sci U S A 95:10576–10578
13. Wondrak G, Roberts M, Cervantes-Laurean D, Jacobson M, Jacobson E (2003) Proteins of the extracellular matrix are sensitizers of photo-oxidative stress in human skin cells. J Invest Dermatol 121:578–586
14. Wondrak G, Roberts M, Jacobson M, Jacobson E (2002) Photosensitized growth inhibition of cultured human skin cells: mechanisms and suppression of oxidative stress from solar irradiation of glycated proteins. J Invest Dermatol 119:489–498
15. Masaki H, Okano Y, Sakurai H (1997) Generation of active oxygen species from advanced glycation end-products (AGE) under ultraviolet light A (UVA) irradiation. Biochem Biophys Res Commun 235:306–310
16. Yasui H, Sakurai H (2000) Chemiluminescent detection and imaging of reactive oxygen species in live mouse skin exposed to UVA. Biochem Biophys Res Commun 269:131–136
17. Warmuth I, Harth Y, Matsui M, Wang N, DeLeo V (1994) Ultraviolet radiation induces phosphorylation of the epidermal growth factor receptor. Cancer Res 54:374–376
18. Sachsenmaier C, Radler-Pohl A, Zinck R, Nordheim A, Herrlich P, Rahmsdorf H (1994) Involvement of growth factor receptors in the mammalian UVC response. Cell 78:963–972
19. Coffer P, Burgering B, Peppelenbosch M, Bos J, Kruijer W (1995) UV activation of receptor tyrosine kinase activity. Oncogene 11:561–569
20. Knebel A, Rahmsdorf H, Ullrich A, Herrlich P (1996) Dephosphorylation of receptor tyrosine kinases as target of regulation by radiation, oxidants or alkylating agents. EMBO J 15:5314–5325
21. Rosette C, Karin M (1996) Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 274:1194–1197
22. Fisher G, Voorhees J (1998) Molecular mechanisms of photoaging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce AP-1-regulated matrix metalloproteinases that degrade human skin in vivo. J Invest Dermatol Symp Proc 3:61–68
23. Persson C, Sjöblom T, Groen A, Kappert K, Engström U, Hellman U, et al (2004) Preferential oxidation of the second phosphatase domain of receptor-like PTP-α revealed by an antibody against oxidized protein tyrosine phosphatases. Proc Natl Acad Sci U S A 101:1886–1891
24. Meng T-C, Fukada T, Tonks N (2002) Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Mol Cell 9:387–399

25. Denu J, Tanner K (1998) Specific and reversible inactivation of protein tyrosine phosphatases by hydrogen peroxide: evidence for a sulfenic acid intermediate and implications for redox regulation. Biochemistry 37:5633–5642
26. Gulati P, Markova B, Göttlicher M, Böhmer F, Herrlich P (2004) UVA inactivates protein tyrosine phosphatases by calpain-mediated degradation. EMBO Rep 5:812–817
27. Fisher G, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S, et al (2002) Mechanisms of photoaging and chronological skin aging. Arch Dermatol 138:1462–1470
28. Fisher G, Talwar H, Lin J, McPhillips F, Wang Z, Li X, et al (1998) Retinoic acid inhibits induction of c-Jun protein by ultraviolet radiation that occurs subsequent to activation of mitogen-activated protein kinase pathways in human skin in vivo. J Clin Invest 101:1432–1440
29. Fisher G, Datta S, Talwar H, Wang Z, Varani J, Kang S, et al (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379:335–339
30. Fisher G, Datta S, Wang Z, Li X, Quan T, Chung J, et al (2000) c-Jun-dependent inhibition of cutaneous procollagen transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. J Clin Invest 106:663–670
31. Fisher G, Wang Z, Datta S, Varani J, Kang S, Voorhees J (1997) Pathophysiology of premature skin aging induced by ultraviolet light. New Engl J Med 337:1419–1428
32. Kang S, Chung J, Lee J, Fisher G, Wan Y, Duell E, et al (2003) Topical N-acetyl cysteine and genistein prevent ultraviolet-light-induced signaling that leads to photoaging in human skin in vivo. J Invest Dermatol 120:835–841
33. Bender K, Göttlicher M, Whiteside S, Rahmsdorf H, Herrlich P (1998) Sequential DNA damage-independent and -dependent activation of NF-κB by UV. EMBO J 17:5170–5181
34. Li N, Karin M (1998) Ionizing radiation and short wavelength UV activate NF-κB through two distinct mechanisms. Proc Natl Acad Sci U S A 95:13012–13017
35. Wu S, Tan M, Hu Y, Wang J-L, Scheuner D, Kaufman R (2004) Ultraviolet light activates NFκB through translational inhibition of IκBα synthesis. J Biol Chem 279:34898–34902
36. Huang T, Feinberg S, Suryanarayanan S, Miyamoto S (2002) The zinc finger domain of NEMO is selectively required for NF-κB activation by UV radiation and topoisomerase inhibitors. Mol Cell Biol 22:5813–5825
37. Park K-J, Gaynor R, Kwak Y (2003) Heat shock protein 27 association with the IκB kinase complex regulates tumor necrosis factor α-induced NF-κB activation. J Biol Chem 278:35272–35278
38. Yamamoto Y, Gaynor R (2001) Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J Clin Invest 107:135–142
39. Csiszar K (2001) Lysyl oxidases: a novel multifunctional amine oxidase family. Prog Nucleic Acid Res Mol Biol 70:1–32
40. Sternlicht M, Werb Z (2001) How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol 17:463–516
41. Liska D, Slack J, Bornstein P (1990) A highly conserved intronic sequence is involved in transcriptional regulation of the α1(I) collagen gene. Cell Regul 1:487–498
42. Chung K-Y, Agarwal A, Uitto J, Mauviel A (1996) An AP-1 binding sequence is essential for regulation of the human α2(I) collagen promoter activity by transforming growth factor-β. J Biol Chem 271:3272–3278
43. Quan T, He T, Voorhees J, Fisher G (2001) Ultraviolet irradiation blocks cellular responses to transforming growth factor-β by down-regulating its type-II receptor and inducing Smad7. J Biol Chem 276:26349–26356
44. Quan T, He T, Kang S, Voorhees J, Fisher G (2004) Solar ultraviolet irradiation reduces collagen in photoaged human skin by blocking transforming growth factor-β type II receptor/Smad signaling. Am J Pathol 165:741–751
45. Griffiths C, Russman A, Majmudar G, Singer R, Hamilton T, Voorhees J (1993) Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). New Engl J Med 329:530–535
46. Talwar H, Griffiths C, Fisher G, Hamilton T, Voorhees J (1995) Reduced type I and type III procollagens in photodamaged adult human skin. J Invest Dermatol 105:285–290
47. Varani J, Spearman D, Perone P, Fligiel S, Datta S, Wang Z, et al (2001) Inhibition of type I procollagen synthesis by damaged collagen in photoaged skin and by collagenase-degraded collagen in vitro. Am J Pathol 158:931–942
48. Fligiel S, Varani J, Datta S, Kang S, Fisher G, Voorhees J (2003) Collagen degradation in aged/photodamaged skin in vivo and after exposure to matrix metalloproteinase-1 in vitro. J Invest Dermatol 120:842–848
49. Varani J, Perone P, Fligiel S, Fisher G, Voorhees J (2002) Inhibition of type I procollagen production in photodamage: correlation between presence of high molecular weight collagen fragments and reduced procollagen synthesis. J Invest Dermatol 119:122–129

50. Mauch C, Hatamochi A, Scharffetter K, Krieg T (1988) Regulation of collagen synthesis in fibroblasts within a three-dimensional collagen gel. Exp Cell Res 178:493–503
51. Varani J, Scuger L, Dame M, Leonard C, Fligiel S, Kang S, et al (2004) Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. J Invest Dermatol 122:1471–1479
52. Roos T, Jugert F, Merk H, Bickers D (1998) Retinoid metabolism in the skin. Pharmacol Rev 50:315–333
53. White J, Guo Y-D, Baetz K, Beckett-Jones B, Bonasoro J, Hsu K, et al (1996) Identification of the retinoic acid-inducible all-trans-retinoic acid 4-hydroxylase. J Biol Chem 271:29922–29927
54. Duell E, Kang S, Voorhees J (1996) Retinoic acid isomers applied to human skin in vivo each induce a 4-hydroxylase that inactivates only trans retinoic acid. J Invest Dermatol 106:316–320
55. Kurlandsky S, Duell E, Kang S, Voorhees J, Fisher G (1996) Auto-regulation of retinoic acid biosynthesis through regulation of retinol esterification in human keratinocytes. J Biol Chem 271:15346–15352
56. Fisher G, Talwar H, Xiao J-H, Datta S, Reddy A, Gaub M-P, et al (1994) Immunological identification and functional quantification of retinoic acid and retinoid X receptor proteins in human skin. J Biol Chem 269:20629–20635
57. Elder J, Fisher G, Zhang Q-Y, Eisen D, Krust A, Kastner P, et al (1991) Retinoic acid receptor gene expression in human skin. J Invest Dermatol 96:425–433
58. Allenby G, Bocquel M-T, Saunders M, Kazmer S, Speck J, Rosenberger M, et al (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. Proc Natl Acad Sci U S A 90:30–34
59. Chambon P (1994) The retinoid signaling pathway: molecular and genetic analyses. Semin Cell Biol 5:115–125
60. Chen S, Ostrowski J, Whiting G, Roalsvig T, Hammer L, Currier S, et al (1995) Retinoic acid receptor gamma mediates topical retinoid efficacy and irritation in animal models. J Invest Dermatol 104:779–783
61. DiSepio D, Sutter M, Johnson A, Chandraratna R, Nagpal S (1999) Identification of the AP1-antagonism domain of retinoic acid receptors. Mol Cell Biol Res Commun 1:7–13
62. Nyirady J, Bergfeld W, Ellis C, Levine N, Savin R, Shavin J, et al (2001) Tretinoin cream 0.02% for the treatment of photodamaged facial skin: a review of 2 double-blind clinical studies. Cutis 68:135–142
63. Bhawan J, Olsen E, Lufrano L, Thorne E, Schwab B, Gilchrest B (1996) Histologic evaluation of the long term effects of tretinoin on photodamaged skin. J Dermatol Sci 11:177–182
64. Ellis C, Weiss J, Hamilton T, Headington J, Zelickson A, Voorhees J (1990) Sustained improvement with prolonged topical tretinoin (retinoic acid) for photoaged skin. J Am Acad Dermatol 23:629–637
65. Rafal E, Griffiths C, Ditre C, Finkel L, Hamilton T, Ellis C, et al (1992) Topical tretinoin (retinoic acid) treatment for liver spots associated with photodamage. N Engl J Med 326:368–374
66. Yamamoto O, Bhawan J, Solares G, Tsay A, Gilchrest B (1995) Ultrastructural effects of topical tretinoin on dermo-epidermal junction and papillary dermis in photodamaged skin. A controlled study. Exp Dermatol 4:146–154
67. Chen M, Goyal S, Cai X, O'Toole E, Woodley D (1997) Modulation of type VII collagen (anchoring fibril) expression by retinoids in human skin cells. Biochim Biophys Acta 1351:333–340
68. Maddin S, Lauharanta J, Agache P, Burrows L, Zultak M, Bulger L (2000) Isotretinoin improves the appearance of photodamaged skin: results of a 36-week, multicenter, double-blind, placebo-controlled trial. J Am Acad Dermatol 42:56–63
69. Armstrong R, Lesiewicz J, Harvey G, Lee L, Spoehr K, Zultak M (1992) Clinical panel assessment of photodamaged skin treated with isotretinoin using photographs. Arch Dermatol 128:352–356
70. Sendagorta E, Lesiewicz J, Armstrong R (1992) Topical isotretinoin for photodamaged skin. J Am Acad Dermatol 27:S15–S18
71. Saurat J, Didierjean L, Masgrau E, Piletta P, Jaconi S, Chatellard-Gruaz D, et al (1994) Topical retinaldehyde on human skin: biologic effects and tolerance. J Invest Dermatol 103:770–774
72. Creidi P, Vienne M-P, Ochonisky S, Lauze C, Turlier V, Lagarde J-M, et al (1998) Profilometric evaluation of photodamage after topical retinaldehyde and retinoic acid treatment. J Am Acad Dermatol 39:960–965
73. Kang S, Duell E, Fisher G, Datta S, Wang Z, Reddy A, et al (1995) Application of retinol to human skin in vivo induces epidermal hyperplasia and cellular retinoid binding proteins characteristic of retinoic acid but without measurable retinoic acid levels or irritation. J Invest Dermatol 105:549–556
74. Kurlandsky S, Xiao J-H, Duell E, Voorhees J, Fisher G (1994) Biological activity of all-trans retinol requires metabolic conversion to all-trans retinoic acid and is mediated through activation of nuclear retinoid receptors in human keratinocytes. J Biol Chem 269:32821–32827
75. Green C, Orchard G, Cerio R, Hawk J (1998) A clinicopathological study of the effects of topical retinyl propionate cream in skin photoageing. Clin Exp Dermatol 23:162–167
76. Kligman L, Sapadin A, Schwartz E (1996) Peeling agents and irritants, unlike tretinoin, do not stimu-

late collagen synthesis in the photoaged hairless mouse. Arch Dermatol Res 288:615–620
77. Kim H, Bogdan N, D'Agostaro L, Gold L, Bryce G (1992) Effect of topical retinoic acids on the levels of collagen mRNA during the repair of UVB-induced dermal damage in the hairless mouse and the possible role of TGF-β as a mediator. J Invest Dermatol 98:359–363
78. Glick A, Flanders K, Danielpour D, Yuspa S, Sporn M (1989) Retinoic acid induces transforming growth factor-β2 in cultured keratinocytes and mouse epidermis. Cell Regul 1:87–97
79. Mitchell R (1967) Chronic solar dermatosis: a light and electron microscopic study of the dermis. J Invest Dermatol 48:203–220
80. Bernstein E, Underhill C, Hahn P, Brown D, Uitto J (1996) Chronic sun exposure alters both the content and distribution of dermal glycosaminoglycans. Br J Dermatol 135:255–262
81. Watson R, Craven N, Kang S, Jones C, Kielty C, Griffiths C (2001) A short-term screening protocol, using fibrillin-1 as a reporter molecule, for photoaging repair agents. J Invest Dermatol 116:672–678
82. King I (1984) Increased epidermal hyaluronic acid synthesis caused by four retinoids. Br J Dermatol 110:607–608
83. Tammi R, Ripellino J, Margolis R, Maibach H, Tammi M (1989) Hyaluronate accumulation in human epidermis treated with retinoic acid in skin organ culture. J Invest Dermatol 92:326–332
84. Elder J, Åström A, Pettersson U, Tavakkol A, Griffiths C, Krust A, et al (1992) Differential regulation of retinoic acid receptors and binding proteins in human skin. J Invest Dermatol 98:673–679
85. Darmon M, Blumenberg M (1993) Retinoic acid in epithelial and epidermal differentiation. In: Darmon M, Blumenberg M (eds) Molecular biology of the skin. Academic Press, San Diego, pp 181–206
86. Duell E, Åström A, Griffiths C, Chambon P, Voorhees J (1992) Human skin levels of retinoic acid and cytochrome P-450-derived 4-hydroxyretinoic acid after topical application of retinoic acid in vivo compared to concentrations required to stimulate retinoic acid receptor-mediated transcription in vitro. J Clin Invest 90:1269–1274
87. Varani J, Ziegler M, Dame M, Kang S, Fisher G, Voorhees J, et al (2001) Heparin-binding epidermal-growth factor-like growth factor activation of keratinocyte ErbB receptors mediates epidermal hyperplasia, a prominent side-effect of retinoid therapy. J Invest Dermatol 117:1335–1341
88. Fisher G, Esmann J, Griffiths C, Talwar H, Duell E, Hammerberg C, et al (1991) Cellular, immunologic and biochemical characterization of topical retinoic acid-treated human skin. J Invest Dermatol 96:699–707
89. Chapellier B, Mark M, Messaddeq N, Calléja C, Warot X, Brocard J, et al (2002) Physiological and retinoid-induced proliferations of epidermis basal keratinocytes are differently controlled. EMBO J 21:3402–3413
90. Xiao J-H, Feng X, Di W, Peng Z-H, Li L-A, Chambon P, et al (1999) Identification of heparin-binding EGF-like growth factor as a target in intercellular regulation of epidermal basal cell growth by suprabasal retinoic acid receptors. EMBO J 18:1539–1548
91. Chen J, Penco S, Ostrowski J, Balaguer P, Pons M, Starrett J, et al (1995) RAR-specific agonist/antagonists which dissociate transactivation and AP1 transrepression inhibit anchorage-independent cell proliferation. EMBO J 14:1187–1197
92. Thacher S, Standeven A, Athanikar J, Kopper S, Castilleja O, Escobar M, et al (1997) Receptor specificity of retinoid-induced epidermal hyperplasia: effect of RXR-selective agonists and correlation with topical irritation. J Pharmacol Exp Ther 282:528–534
93. Fluhr J, Vienne M, Laure C, Dupuy P, Gehring W, Gloor M (1999) Tolerance profile of retinol, retinaldehyde and retinoic acid under maximized and long-term clinical conditions. Dermatology 199:57–60
94. Latriano L, Tzimas G, Wong F, Willis R (1997) The percutaneous absorption of topically applied tretinoin and its effect on endogenous concentrations of tretinoin and its metabolites after single doses or long-term use. J Am Acad Dermatol 36:S77–S85
95. Jick S, Terris B, Jick H (1993) First trimester topical tretinoin and congenital disorders. Lancet 341:1181–1182
96. Lipson A, Collins F, Webster W (1993) Multiple congenital defects associated with maternal use of topical tretinoin. Lancet 341:1352–1353