Retinoic Acid Receptors and Topical Acne Therapy: Establishing the Link Between Gene Expression and Drug Efficacy

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Topical retinoids have been an integral part of the dermatology armamentarium for 3 decades. Tretinoin (all-trans-retinoic acid), a naturally occurring retinoid compound, was approved and released in the United States in 1971 for acne therapy. Several strengths and vehicles (cream, gel, solution) of tretinoin became available to titrate use based on patient skin type, efficacy, and tolerance. Two synthetic retinoids have been approved and released for use in the United States over the past 5 years. Adapalene, a naphthoic acid derivative with retinoid activity, was released in 1996 for acne therapy. Tazarotene followed in 1997, with initial approval for psoriasis and more recent approval for acne treatment. Despite the success and widespread application of prescription-based topical retinoids used for acne and other skin disorders and retinol as a component of various cosmetics, little was known about the mechanism of action of these compounds until the last decade. As the use of retinoids (especially systemic agents) demonstrated significant therapeutic impact for several severe diseases in both dermatology and other disciplines (eg, oncology), interest in understanding retinoid cellular mechanisms intensified. In late 1987 and early 1988, researchers identified specific nuclear retinoic acid receptors (RARs), suggesting important insights into the cellular/molecular basis of retinoid activity. RARs have been found in several organ tissues, including skin. With further knowledge of retinoid cellular mechanisms, researchers perceived the potential for developing new effective and safe retinoid compounds with a better understanding of their selective activity against specific disease states.

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Updated Definition: What Is a Retinoid?

The term *retinoid* currently includes both naturally occurring and synthetic compounds that may or may not be structurally related to vitamin A (retinol).[1-3] An international joint commission report on biochemical nomenclature in 1982 initially stipulated that a retinoid must be related chemically/structurally to vitamin A. Since then, several compounds exhibiting biologic retinoid activity that are not related structurally to vitamin A have been synthesized and studied in vitro and in vivo, including use in humans. An updated and expanded definition of "retinoid" has been suggested, taking into account the elicitation of specific biologic responses.[2] The definition describes a retinoid as "a substance that can elicit specific biologic responses by binding to or activating a specific receptor or set of receptors. Retinoids are those substances that have a specific molecular fit to the receptor; they are defined in terms of their interaction with that receptor, rather than being restricted to a particular subset of diterpenoid, polyene substances."[2] This definition is more applicable to modern reality and current directions in retinoid research. "The program for the biological response of target cells for retinoids resides in retinoid receptors, rather than in the retinoids themselves."[2]

Retinoic Acid Receptor Genes and Pathways of Activation

Retinoic Acid Receptors—In the mid-to-late 1980s, independent investigators identified 2 distinct retinoic acid receptors (RARs), RAR-α and RAR-β,

both of which were found to express low levels of the RAR-α and RAR-β genes in adult human skin.[4] This confounded researchers because skin was well-known to be highly responsive to the effects of retinoid, especially based on clinical and research experience with topical tretinoin and oral isotretinoin. The later discovery of RAR-γ in both human and murine skin in 1989 appeared to clarify the controversy, as this specific receptor gene was expressed in skin at high levels.[4,5]

RAR-α, RAR-β, and RAR-γ are the 3 distinct RAR genes identified in humans. Gene transcripts for RAR-α are relatively ubiquitous in adult and embryonic tissues.[1,4] The RAR-α gene is expressed at a higher degree in fetal skin than adult skin in vivo and in human neonatal keratinocytes in vitro. Such findings suggest that RAR-α most likely plays an important role in overall cellular growth and differentiation.[1,4]

The RAR-β gene appears to play a limited role in adult skin.[1,4] To date, studies suggest that RAR-β gene expression has been dependent on the cell type analyzed. Induction of RAR-β by retinoic acid has been demonstrated in dermal fibroblasts, with a delayed kinetic pattern and with more rapid onset in both hepatoma and teratocarcinoma cell lines, but not in keratinocytes.[4,6]

The most abundant and readily detectable RAR type in adult skin is RAR-γ.[4,5] Based on in vitro and in vivo data, including data from studies in human skin, the RAR-γ gene appears to be abundantly expressed in human skin as compared with RAR-α and RAR-β and is expressed in both human keratinocytes and dermal fibroblasts.[4,5] Retinoid binding and agonist activation of RARs have been demonstrated to modulate gene expression through retinoid (retinoic acid) response elements (RAREs) of DNA.[3] The specific products of such gene modulation/expression, apparently influenced by the role of individual RARs (and probably other yet-to-be-discovered receptors), create the varied biologic effects of retinoids.

Different topical retinoids may vary in their route of cellular transport; use of cellular-binding proteins, such as cellular (cytoplasmic) retinoic acid-binding protein (CRABP); RAR binding patterns; and RAR binding affinity.[4,7,8] The nature of both therapeutic and adverse retinoid effects produced by a specific topical retinoid depends on the pattern and affinity of receptor binding and the multiplicity of pathways for receptor-gene activation. In addition, as isoforms of RARs have been identified, this also may relate to specific functions and resultant clinical effects. At least 7 isoforms of RAR-γ have been described.[4,6]

Additional in vivo animal research with recognized models (Rhino mouse and rabbit) using multiple RAR-γ–selective retinoids suggests that (1) RAR-γ plays a significant role in retinoid efficacy, and (2) that retinoid-induced skin irritation may be mediated at least in part by receptor interaction.[4,5] In a study of multiple retinoid agents using a mouse model, retinoid-induced skin irritation was correlated directly to receptor-binding affinity for RAR-β, RAR-γ, or both.[9] Cutaneous irritation was not related to RAR-α agonist activity. The study also reported that in regard to skin irritation, a distinction could not be made between the relative contributions of RAR-β and RAR-γ receptor binding.[9]

Retinoid X Receptors—In 1990, another receptor type, the retinoid X receptor (RXR), was discovered.[1,4] Three types appear to exist: RXR-α, RXR-β, and RXR-γ. Of the RXRs, RXR-α expression appears to be predominant in human epidermis. None of the currently available prescription or over-the-counter retinoids bind directly to RXRs. However, conversion to 9-*cis*-retinoic acid is one of the intracellular metabolic pathways of all-*trans*-retinoic acid; up to 5% epidermal recovery of 9-*cis*-retinoic acid has been reported after application of a topical retinoic acid. 9-*cis*-Retinoic acid may bind with high affinity to RARs and also serves as a ligand for RXR binding.[1,3,4]

RARs and RXRs exhibit considerable cooperative activity in binding to DNA.[3] After retinoid binding and activation, RARs require the formation of a heterodimer complex with a bound RXR before interaction with DNA. To the contrary, RXRs may behave as homodimers or form complexes with other nonretinoid hormone receptors (eg, thyroid).

Other than the role of RXR in RAR-RXR heterodimer formation required for DNA interaction and gene expression, the direct role of RXRs in the therapeutic or adverse effects of topical retinoids is unknown. An in vivo study in the hairless mouse model comparing topically applied RAR-specific and RXR-selective retinoids suggests that RXRs behave primarily as "silent partners," functioning passively in the RAR-RXR heterodimer complex.[10] In contrast to RAR-specific retinoids, RXR-selective retinoids produced only very mild evidence of skin irritation (flaking) and demonstrated weak potency in induction of epidermal hyperplasia. Further research is needed to better define the cellular and biologic activities of RXRs.

Cellular Pathways of Activity: Topical Retinoids

The receptor-binding activities of topical retinoids currently approved for use in the United States

RAR Binding Properties of Topical Retinoids*[1,7,11]

Drug	CRABP	RAR-α	RAR-β	RAR-γ	RXR
Tretinoin (all-*trans*-retinoic acid)	+	+	+	+	–[†]
Adapalene	–	+/–	+	+	–
Tazarotene[‡]	?	+/–	+	+	–

*RAR indicates retinoic acid receptor; CRABP, cellular (cytoplasmic) retinoic acid-binding protein; RXR, retinoid X receptor; +/–, weak binding.
†Tretinoin does not exhibit direct binding to RXR; isomerase enzymatic conversion of tretinoin to 9-*cis*-retinoic acid results in binding with RXR.
‡Tazarotene is a prodrug; tazarotenic acid is the active metabolite of tazarotene.

are outlined in the Table. Gene expression requires (1) gaining access to the cytoplasm after topical application, followed by (2) intracellular binding with cytoplasmic proteins involved in retinoid transport, (3) binding with nuclear receptors (eg, RARs, RXRs), and (4) interaction with genes possessing specific promoter-region DNA sequences called *RAREs*.[3]

The cellular pathway sequence has been studied intensively and reasonably well-defined for all-*trans*-retinoic acid, with induction of several genes bearing RARE demonstrated both in vivo and in vitro.[3,7] As a naturally occurring retinoid, all-*trans*-retinoic acid uses inherent cellular mechanisms for transport and metabolism.

It is not correct to assume that other retinoids use the same transport mechanisms and binding patterns as all-*trans*-retinoic acid. For example, unlike all-*trans*-retinoic acid, adapalene does not appear to bind to CRABPs during cellular transport.[7] This is not surprising because adapalene is not a naturally occurring retinoid; a synthetic agent would be less likely to use an inherent cellular mechanism of retinoid transport or metabolism. Available in vitro data performed using cultured human keratinocytes have shown that binding of CRABPs is not required to produce biologic retinoid effects, such as keratinocyte differentiation.[8] This study also suggests that CRABPs do not play a qualitative role in retinoid activity but may be active in regulating the quantity of intracellular retinoic acid or may function as part of a retinoic acid feedback mechanism.

Tazarotenic acid, the active metabolite of tazarotene, also demonstrates some variation in its biologic pathway sequence and appears not to be convertible to any other potentially active retinoid forms.[12] In addition to RAR-β and RAR-γ binding, tazarotenic acid reduces abnormal expression of epidermal growth factor and keratinocyte transglutaminase I and downregulates gene expression dependent on activator protein-1, a transcription factor associated with cell proliferation and inflammation (negative gene regulation).[7] These cellular activities are believed to relate to the role of tazarotene in psoriasis treatment.

Conclusion

Because of a significant increase in the understanding of cellular mechanisms, the definition of a retinoid has been modified to include the concepts of retinoid receptors and biologic activity. Within approximately one decade, the discovery of RARs and their influence on gene expression have revolutionized retinoid research. Since the initial availability of tretinoin in 1971, topical retinoids continue to experience widespread use because of high efficacy in disorders such as acne, as well as other applications. As further research better defines cause-and-effect patterns related to specific RAR interactions, newer topical and systemic retinoid agents are likely to be developed, and expanded uses undoubtedly will emerge. This may include selective and pan-receptor agonists designed to modulate specific therapeutic effects, and selective antagonists used to inhibit specific adverse reactions, such as cutaneous or mucosal irritation.

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CONTINUED ON PAGE 139

CONTINUED FROM PAGE 129

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