Expression of Retinoid Nuclear Receptor Superfamily Members in Human Hair Follicles and Its Implication in Hair Growth

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Since clinical evidence of hair loss and hair depigmentation following etretinate therapy has been reported, we decided to study the expression levels of several members of the retinoid nuclear receptor superfamily in dermal and epithelial compartments of the human hair follicle. Additionally, we evaluated the effects of several ligands for these receptors on human hair growth in culture in vitro. We observed that the cellular/cytoplasmic retinoic acid (RA) binding protein-II and the retinoid-X-receptor-α were constantly and strongly expressed in both compartments at levels comparable to those of vitamin D receptor. In dermal papilla cells, by contrast with RARβ which was always expressed, RARα and RARγ were not constantly expressed. In dermal sheath fibroblasts, both RARα, RARβ and RARγ mRNAs were moderately expressed, while in the epithelial compartment, namely the plucked hair, we observed the expression of the same genes in the absence of RARβ. We also observed that RAR agonists all-*trans* RA and CD367 inhibited the survival of human hair follicles in culture in vitro, while RXR agonist CD2425 stimulated hair growth and survival at levels comparable to those of 1α,25-dihydroxyvitamin D_3, suggesting that RXR agonists might stimulate hair growth in humans in vivo. *Key words: Vitamin A; RAR; RXR; VDR; RT/PCR.*

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Vitamin A and its derivatives such as retinoic acid (RA) are known to exert pleiotropic effects on basic biological programs (1–3 for recent reviews). They are also believed to play an important role during morphogenesis and embryological development of hair follicles, as shown on mouse vibrissa (4, 5). Since the hair growth cycle, which involves interaction between the mesenchyme-derived dermal papilla cells and the epithelial components of the hair follicle (6), appears to partially recapitulate the embryogenesis of the hair follicle, RA may well be involved also in the control of the hair cycle.

Two classes of specific receptors for RA have been described (3, 7, 8 for reviews). One class consists of both the nuclear retinoic acid receptors (RAR)s and the nuclear retinoid-X receptors RXR(s). While 9-*cis* RA is the preferential ligand of RXR(s), both all-*trans* and 9-*cis* RA bind with high affinity to RARs (8). These ligand(s)-activated nuclear receptors heterodimerize (or alternatively homodimerize) to act as transcriptional activators (or repressors) by binding to specific DNA sequences (RA-responsive elements, RARE) in the regulatory regions of several target genes (3, 7–10). The other class of specific receptors for RA includes cellular retinoic acid-binding proteins (CRABP)s that may be involved in the transport/sequestration and metabolism of retinoids in the cytoplasm (see 11, 12 for review).

As an initial step to determine the role of RA in homeostasis of hair follicles, we investigated the mRNA expression levels for RARα, RARβ, RARγ, RXRα and CRABP-II in the different compartments of the pilosebaceous unit, using reverse transcriptase polymerase-chain reaction (RT/PCR).

As a second step, we investigated the effects of RAR agonist and RXR agonist ligands, by comparison with 1α,25-dihydroxyvitamin D_3 (vitamin D3) on the growth of human hair follicles in vitro.

MATERIALS AND METHODS

Chemicals

Vitamin D3 (Cat No 154300) was purchased from ICN (Aurora, Ohio). RA (all-*trans* RA), the CD367 RAR pan-agonist 4-(5, 6, 7, 8-Tetrahydro-5, 5, 8, 8-tetramethyl-2-anthracenyl) benzoic acid (13) and the CD2425 RXR agonist 2-[(E)-2-(3, 5, 5, 8, 8- pentamethyl-5, 6, 7, 8- tetrahydro-2-naphtyl)-propen-1-yl]-4-thiophene carboxylic acid (14) were kindly provided by CIRD Galderma (Sophia Antipolis, France).

Molecular weight markers: M1: 50–2000bp ladder (Cat No 170–8200) was purchased from Bio-Rad (Hercules, California), and M2: 100 base pair ladder (Cat N°27–4001–01) was purchased from Pharmacia Biotech (Uppsala, Sweden).

Plucked hair follicles

Five anagen hairs were individually and gently plucked from the vertex of male volunteers. They were immediately soaked in William's E medium (Gibco BRL Bethesda, MD) and rapidly observed under a microscope to check for the presence of an intact outer root sheath, as well as for the absence of dermal papilla and sebaceous gland. As reported (15), plucked hair consists exclusively of the upper two thirds of the hair follicle (i.e. mainly ORS and inner root sheath keratinocytes without their perifollicular external connective layer).

Human hair follicle isolation and culture in vitro

Individual human terminal scalp hair follicles from facelift surgery (exclusively from female patients) were isolated as follows: the sample was cut in several strips of approximately 5 × 10 mm and put upside-down in a Petri box. The fat subcutaneous layer was then taken out with fine forceps, so that the bulb from each anagen follicle could be clearly identified. Then, each follicle was plucked from the dermal-epithelial junction, without altering the dermal papilla and the matrix cells, so that no sebaceous material remained associated with the hair follicles. Each isolated hair follicle with its perifollicular external connective layer was then incubated at 37°C in a water-saturated atmosphere of 5% CO_2 / 95% air. Hair follicles were maintained free-floating (but not at the air/medium interface) in William's E culture medium, supplemented with 2 mM L-glutamine, 10 µg/ml insulin, 10 ng/ml hydrocortisone and antibiotics, as described (16). The variable survival time for controls is due to inter-individual variations. In fact, hair follicles were obtained from facelift surgery, which did not contain enough follicles to test all compounds at a time on follicles from the same donor. Nevertheless, each representative experiment was performed on 12 follicles from the same donor.

Dermal papilla cell isolation and culture in vitro

Dermal papillae from individually isolated hair follicles were microdissected, as described (17, 18), and cultured in medium 199 containing 2 mM L-glutamine, 1% of antibiotic/antimycotic solution (Gibco BRL Bethesda, MD) and 10% of fetal calf serum (Gibco BRL Bethesda, MD) at 37°C, 5% CO_2.

Connective tissue sheath cell culture

Culture of dermal fibroblasts from human hair follicle connective tissue sheath was seeded at 2.10^5 /60 mm Petri dishes in Dulbecco's Modified Eagle Medium (DMEM), supplemented with L-glutamine (2 mM), 1 mM sodium pyruvate and antibiotics (penicillin-G, 100 Units/ml; streptomycin-S, 100 µg/ml; amphotericin 250ng/ml) in the presence of 10% fetal calf serum Gibco BRL, Bethesda, MD), as previously described (19).

mRNA expression (RT/PCR analysis)

mRNA from either 5 plucked hairs or from $5* 10^6$ dermal papilla cells was prepared using the QuickPrep® mRNA preparation kit (Pharmacia Biotech, Uppsala, Sweden). Poly-A+ mRNA(s) were then reverse transcribed using the first strand cDNA synthesis kit from Pharmacia, and PCR was performed using the Taq polymerase and the 10X buffer marketed by Amersham (Les Ulis, France), as previously described (16, 19, 20), with the following primers purchased from Genset (Paris, France): $hRAR\alpha_1$ (1145 bp): (FP) 5'-CCA GGC GCT CTG ACC ACT CTC C-3'; (RP) 5'-GCC CTC TGA GTT CTC CAA CA-3'. $hRAR\gamma_1$ (1253bp): (FP) 5'-GGC CAC CAA TAA GGA GCG ACT CT-3'; (RP) 5'-GGG TTC TCC AGC ATC TCT CGG-3'. $hRXR\alpha$ (300 bp): (FP) 5'-GCT TGT GTC CAA GAT GCG GG-3'; (RP) 5'-GCA GGC CTA AGT CAT TTG GTG C-3'. hCRABPII (271bp): (FP) 5'-GGC AAC TGG AAA ATC ATC CCG ATC-3'; (RP) 5'-CAC TCT CCC ATT TCA CCA GGC TCT-3'. h β actin (1067bp): (FP) 5'-ATG GAT GAT GAT ATC GCC GCG CT-3'; (RP) 5'-CGG ACT CGT CAT ACT CCT GCT GCT TG-3'. Human vitamin D receptor (VDR) primers (amplimer, 1245 bp) and human $RAR\beta_2$ primers (amplimer, 1300bp) have been described by others (21). GAPDH primers were purchased from Clontech (Palo Alto, CA). 1/15 of the cDNA was used for PCR (1 cycle at 95°C, 4 min, followed by 30 cycles at 95°C, 25 s; 57°C, 1 min, 72°C, 1 min), as previously described (16, 19, 20). Each amplified fragment was then migrated on 2% agarose gel containing 0.5 µg/ml ethidium bromide. Gels were irradiated under a UV transilluminator, and a video camera (Vilbert Lourmat, Marne-la-Vallée, France) captured the image. Using an imaging analyser (Vilbert Lourmat, Marne-la-Vallée, France), we evaluated the relative intensity of each amplified fragment.

Statistical analysis

The significance of differences between untreated follicles and treated follicles was determined using the variance analysis for growth and the logrank test for survival.

RESULTS

mRNA expression levels for RARs, RXRα and CRABPII in compartments of the pilosebaceous unit

In the dermal compartment, using primary cultures of dermal papilla cells, we could detect mRNAs for all RAR(s) as well as for CRABPII, RXRα and VDR (Fig. 1A). However, we noticed variations in the intensity and the frequency of their relative expression: while mRNA expression of both CRABPII and RXRα was strong and constant, RARβ expression levels progressively decreased following passages (Fig. 1B). We also noticed variations in the frequency of RARα and RARγ expression. In fact RARγ could be detected at a high level in only 5 out of 9 individual experiments, while RARα was found

expressed in dermal papilla cells at a high level in 6 out of 9 individual experiments (see Table I). In primary cultures of hair follicle connective tissue sheath cells, all RARs as well as RXR, CRABP-II and VDR were also found expressed (Fig. 1C). Similarly to dermal papilla cells, the expression levels of the three forms of RARs were low compared to those of RXR, CRABP-II and VDR.

By contrast with primary culture of cells from the dermal compartment, RARβ transcript was not detectable in freshly plucked hairs, which indeed represent the epithelial compartment of the hair follicle, mainly constituted of outer and inner root sheath keratinocytes (15) (Fig. 1D). RXRα, CRABP-II and VDR were found to be the predominant receptor transcripts in freshly plucked hair. In this epithelial compartment, RARγ was the strongest form of RAR expressed, while RARα mRNA was found only at a very low level.

Effects of all-trans RA, CD367, CD2425 and vitamin D3 on the growth and survival rate of human hair follicle in vitro

According to these patterns of retinoid nuclear receptors expression, we investigated the effects of some of their respective ligands on the growth rate and survival of freshly dissected human hair follicles grown in vitro. As shown in Fig. 2A, we evidenced that all-*trans* RA treatment (10^{-9} M) induced an abrupt and significant cessation of the survival of human hair follicles ($p = 0.003$ versus control). Compared to the control conditions, in the first 10 days of culture, the growth rate of all-*trans* RA-treated remaining follicles was similar to that of control untreated hair follicles, but at day 11, none of the 12 treated hair follicles were growing anymore (half life = 7 days), while in the control group, 9 out of the 12 hair follicles (75%) were still growing (half life = 26 days). In a separate experiment (Fig. 2B), the RARαβγ pan-agonist CD367 at 10^{-8} M was also found to strongly and significantly inhibit the survival of hair follicles in vitro, compared to the control untreated follicles ($p = 0.0001$ versus control). In fact, at day 6, none of the 12 treated follicles were growing anymore, while in the control group, 10 out of the 12 hair follicles (83%) were still growing.

By contrast with the inhibitory effects of all-*trans* RA and RAR agonist, as shown in Fig. 2C, we confirmed a significant positive effect of 10^{-8} M vitamin D3 on the growth rate of human hair follicles in culture in vitro ($p = 0.001$ versus control) (22). Similarly, as shown in Fig. 2D, the RXR agonist CD2425 dose-dependently stimulated the growth of human hair follicles in vitro compared to the control untreated follicles. This stimulatory effect was observed at relatively low concentrations of the RXR agonist CD2425 (10^{-10} and 10^{-9} M) compared to vitamin D3 and was maximal at 10^{-8} M ($p = 0.003$ versus control). Furthermore, in addition to increasing growth rate of hair follicles in vitro, CD2425 at a concentration of 10^{-8} M was also found to increase the survival of hair follicles in culture (half life control = 26 days; half life CD2425 = 32 days).

DISCUSSION

The expression profiles of the different RARs, RXR, CRABP-II and VDR transcripts were analysed in primary cultures of human hair dermal papilla cells and dermal sheath fibroblasts. Although it cannot be excluded that substantial
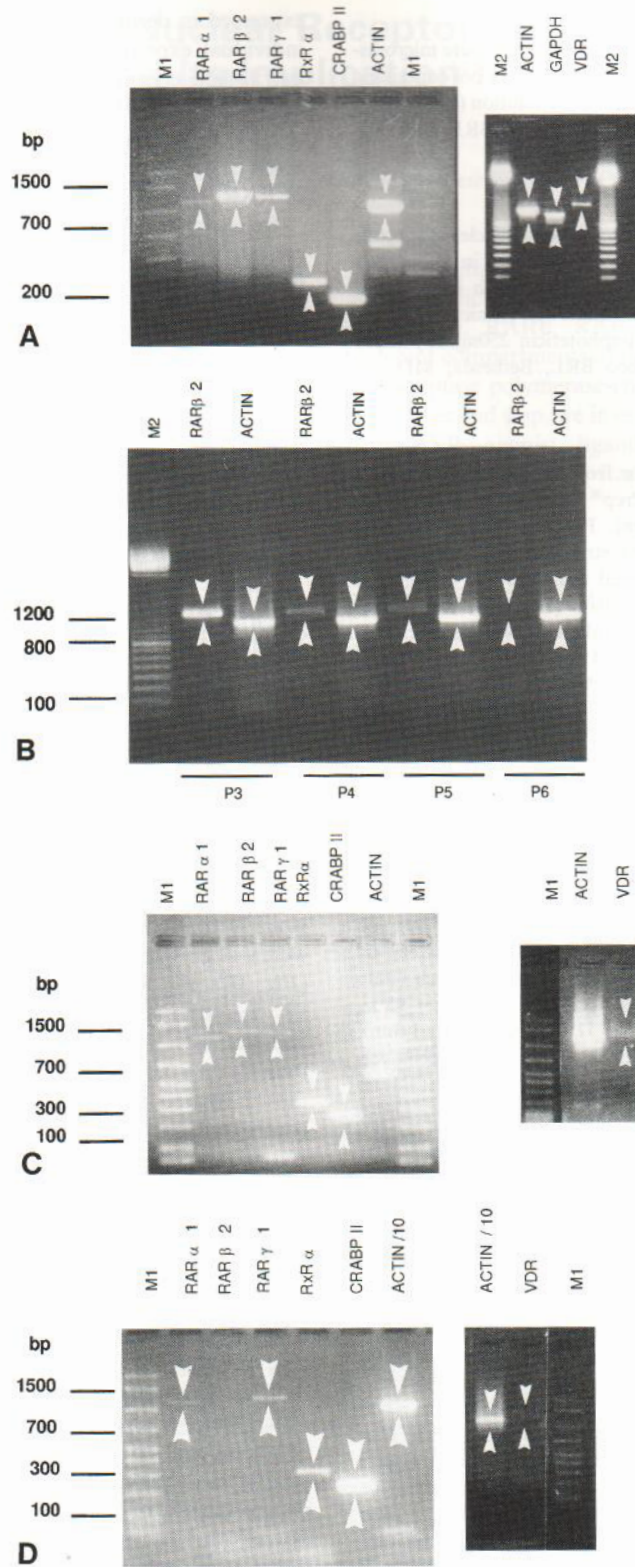
Fig. 1. Semi-quantitative RT/PCR analysis of several members of the non-steroid nuclear receptor superfamily in primary culture of dermal papilla cells (1A), with a progressive decline of RARβ expression in dermal papilla cells following passages (P3 to P6) (1B), in primary culture of connective tissue sheath fibroblasts (1C) and in freshly plucked hairs in culture in vitro (1D). In the dermal compartment (dermal papilla and connective tissue sheath fibroblasts) the major transcripts are CRAPBII, RXRα, VDR and RARβ. RARα and RARγ were also found to be expressed, which is not always the case (see Table I). RARβ expression in dermal papilla cells was strong, since this experiment was performed on an early passage (see Table I). In the epithelial compartment, the major transcripts are CRAPBII, RXRα, VDR and to a lesser extent RARα and RARγ. Note the lack of expression of RARβ in this compartment. Actin and GAPDH (house-keeping genes) were used as controls. In some experiments, cDNA was diluted 10-fold for amplification of actin (actin/10). M_1 and M_2: molecular weight markers. Arrowheads indicate the expected position of the amplified bands.

Table I. *The mRNA expression of RARβ is greater than that of the other nuclear receptors in primary culture of dermal papilla cells*

Passages	RARα	RARβ	RARγ
2	0.25	0.5	0.25
3	0.16	0.79	0.05
3	0.05	0.93	0.02
4	0.02	0.82	0.16
5	0.05	0.93	0.02
5	0.07	0.64	0.29
5	0.05	0.68	0.27
6	0	1	0
9	0	1	0
mean	0.07	0.81	0.12

Amplified fragments (semi-quantitative RT/PCR analysis) were analysed as described in Materials and Methods. The sum of the three fragments (RARα + RARβ + RAR γ) was arbitrarily normalized to 1.00. The value 0.05 was arbitrarily assessed to the lowest limit of detection of mRNA levels.

changes in mRNA expression levels may occur during culture of the cells in vitro, our data indicate that RARβ, RXRα and CRABP-II are the major retinoid receptors probably and constantly involved in RA effects on dermal papilla cells and dermal sheath fibroblasts. Some reports have already indicated the expression of both classes of retinoid receptors in the pilosebaceous unit. In human dermal papilla cells RARβ has also been found by others to be the major transcript, followed by RARα (18). By contrast, RARγ was reported to be only faintly expressed in this compartment (18), and RARβ expression was suggested to be restricted to adults, based on the findings that mouse embryo vibrissae dermal papilla cells expressed only RARα and RARγ during the first two stages of the hair follicle development (4, 5).

In freshly plucked hair follicles, by contrast with cells from the dermal compartment in culture, we did not detect RARβ mRNA. This pattern, which reflects exactly the in vivo situation in the keratinocytes of the inner and of the outer root sheaths at the time of plucking, strongly suggests that RARβ is not involved in the epithelial compartment of the pilosebaceous unit in vivo.

This lack of RARβ mRNA expression and protein detection has already been reported by others in human skin keratomes (23, 24), suggesting that the absence of RARβ is a common feature of these two epithelial compartments. In addition, RARγ was the strongest form of RAR mRNA expressed in plucked hair, while RARα was only slightly expressed.

Furthermore, the human hair follicle responds in vitro to all-*trans* RA, vitamin D3 or RXR agonists, suggesting that the receptors corresponding to the detected mRNAs are functional in the human hair follicle. Two opposite effects could be observed: while the RAR agonists all-*trans* RA (10^{-9} M) and CD367 (10^{-8} M) significantly inhibited hair survival, by contrast, both the RXRα agonist and vitamin D3 significantly stimulated hair growth. However, since we used relatively high concentrations of RAR agonists in our experiments (10^{-8} M – 10^{-9} M), we could not discriminate between RA and CD367 as to their ability to inhibit hair growth (or survival) in vitro. Thus, since CD367 was described as a better transcriptional activator of RARE than RA (13), one could

not exclude that dose-response studies in the [pM-nM] range would show a more potent inhibitory effect of CD367 than RA on hair growth or survival in vitro.

In fact, some reports have already reported that etretinate (another synthetic RAR agonist) alters scalp hair growth in vivo by causing either alopecia (25, 26) or morphological changes (27), as well as transitory defects in hair pigmentation (28). The exact mechanism by which all-*trans* RA mediates its inhibitory effect in vitro is not well elucidated. Since it has been reported that all-*trans* RA induced a strong production of IL-1α and IL-8 by the epithelial cell line HOC-7 (29) and since several reports indicate an inhibitory role of IL-1α and IL-1β on hair growth (16, 30, 31), it is possible that all-*trans* RA inhibits hair survival in vitro through the induction of IL-1 production. Furthermore, we observed that all-*trans* RA (10^{-8} M) had a marked inhibitory effect on the growth of dermal papilla cells in culture in vitro (data not shown), suggesting that the dermal papilla could be one of the other primary targets of all-*trans* RA action.

On the other hand, we confirmed the observation from others (22) showing a positive effect of vitamin D3 (10^{-8} M) on hair growth. In addition, we found here that the RXR agonist CD2425 (10^{-10} to 10^{-8} M) enhanced the survival and growth rate of human hair follicles cultured in vitro. However, this latter observation is not in accordance with recent data from others (32), showing that another RXR agonist (i.e. SR11237) at 10^{-7} M did not stimulate the growth of equine mane hair in culture. This discrepancy might be related either to the intrinsic specificity of equine mane hair, which does not grow for more than 5 days in vitro (32), or to the higher concentration (10^{-7} M) of RXR agonist used. Alternatively, mane follicles might be regulated differently from human hair follicles. Furthermore, different receptor-binding properties regarding other members of the nuclear receptor superfamily, as well as distinct selectivities in terms of heterodimerization between the RXR agonists CD2425 and SR11237, might be another plausible explanation for these different observations.

The present study has shown that the RXR agonist CD2425 stimulates hair growth in vitro. Since RXR is described as an essential accessory factor for heterodimerization and high affinity binding both of RXR/RAR complexes and RXR/VDR heterodimers on their cognate hormone-responsive elements (see 33, 34 for review), we speculate that RXR agonists, e.g. CD2425, may preferentially promote the VDR/RXR pathway rather than the RAR/RXR pathway in human hair follicles by a yet unknown mechanism. Alternatively, it has been reported (34, 35) that the 9-*cis* RA ligand may divert RXR(s) away from VDR- or RAR-dependent pathways to induce homodimer formation of RXR. Following these observations, our data suggest that CD2425 destabilizes RAR/RXR heterodimers by enhancing the formation of either RXR homodimers or RXR/VDR heterodimers. Whether RXR agonists stimulate hair growth in humans in vivo now remains to be established.
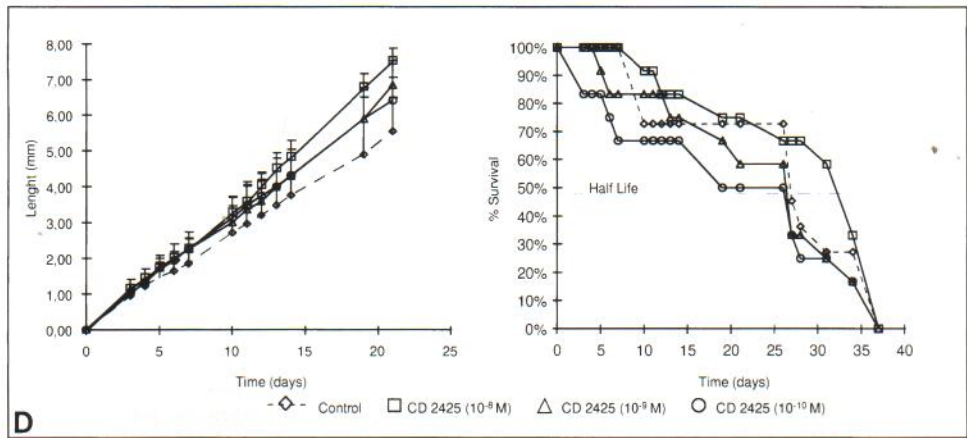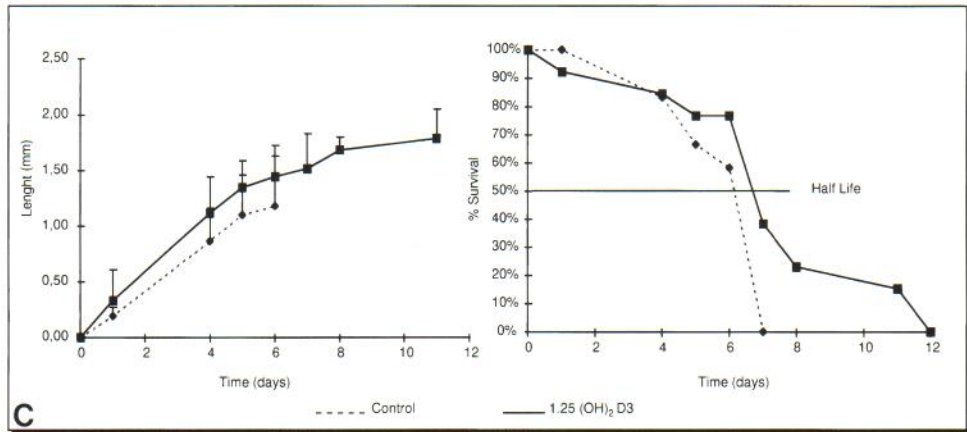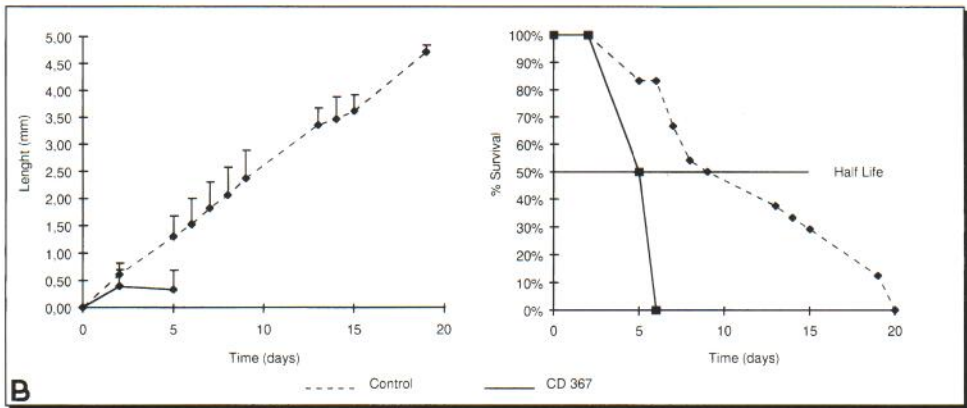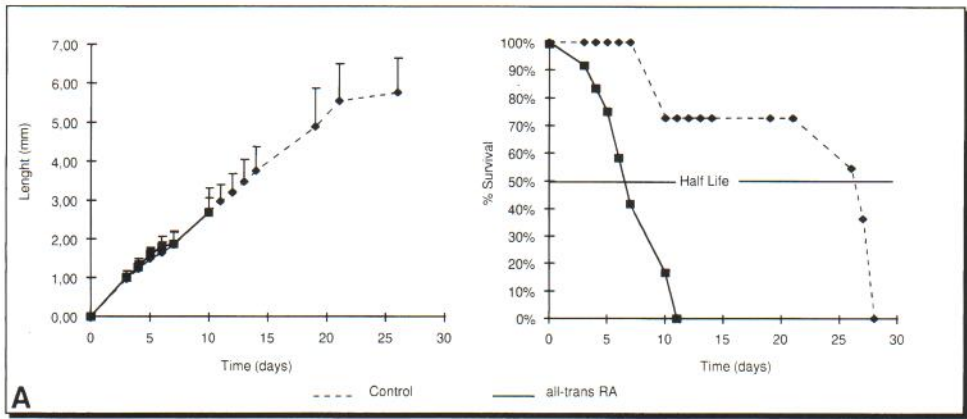
REFERENCES

1. Gudas LG. Retinoïds and vertebrate development. J Biol Chem 1994; 269: 15399–15402.
2. Maden M. The retinoic acid supergun affair. Curr Biol 1994; 4: 281–284.
3. Pfahl M, Chytil F. Regulation of metabolism by retinoic acid and its nuclear receptors. Annu Rev Nutr 1996; 16: 257–283.
4. Viallet JP, Ruberte E, Krust A, Zelent A, Dhouailly D. Expression of retinoic acid receptors and dermal-epidermal interactions during mouse skin morphogenesis. In: Morrisse-Kay G, ed. Retinoids in normal development and teratogenesis. Oxford: Oxford Publ., 1992: 192–210.
5. Viallet JP, Dhouailly D. Retinoic acid and mouse skin morphogenesis, I. Expression pattern of retinoic acid receptors genes during hair vibrissa follicle, plantar, and nasal gland development. J Invest Dermatol 1994; 103: 116–121.
6. Jahoda CAB, Reynolds AJ. Dermal-epidermal interactions-follicle-derived cell populations in the study of hair-growth mechanisms. J Invest Dermatol 1993; 101: 33S-38S.
7. Chambon P. A decade of molecular biology of retinoic acid receptors. FASEB J 1996; 10: 940–954.
8. Allenby G, Janocha R, Kazmer S, Speck J, Grippo JF, Levin AA. Binding of 9-cis-retinoic acid and all-trans-retinoic acid to retinoic acid receptors α, β and γ. J Biol Chem 1995; 269: 16689–16695.
9. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, et al. The nuclear receptor superfamily: the second decade. Cell 1995; 83: 835–839.
10. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. Cell 1995; 83: 841–850.
11. Napoli JL. Retinoic acid biosynthetis and metabolism. FASEB J 1996; 10: 993–1001.
12. Sanquer S, Gilchrest BA. Characterization of human cellular retinoic acid-binding proteins-I and-II: ligand binding affinities and distribution in skin. Arch Biochem Biophys 1994; 311: 86–94.
13. Bernard BA, Bernardon JM, Delescluse C, Martin B, Lenoir MC, Maignan J, et al. Identification of synthetic retinoids with selectivity for human nuclear retinoic acid receptor γ. Biochem Biophys Res Comm 1992; 186: 977–983.
14. Chandraratnan RA, Chandraratnan RAS. Therapeutic use of selective retinoid X receptor agonists-are cell proliferation and differentiation regulators, do not have teratogenic or irritant properties. Patent No WPI 94–279367/34. 1994.
15. Lenoir MC, Bernard BA, Pautrat G, Darmon M, Shroot B. Outer root sheath cells of human hair follicle are able to regenerate a fully differentiated epidermis in vitro. Dev Biol 1988; 130: 610–620.
16. Mahé YF, Buan B, Billoni N, Loussouarn G, Michelet JF, Bernard BA. Pro-inflammatory cytokines cascade in human plucked hair. Skin Pharmacol 1996; 9: 366–375.
17. Messenger AG. The culture of dermal papilla cells from human hair follicle. Br J Dermatol 1984; 110: 685–689.
18. Randall VA, Thornton MJ, Hamada K, Redfern CPF, Nutbrown M, Ebling FJG, Messenger AG. Androgens and the hair follicle; cultured dermal papilla cells as a model system. Annals NY Acad Sci 1991; 642: 355–375.
19. Mahé YF, Buan B, Bernard BA. A minoxidil-related compound lacking a C6 substitution still exhibits strong anti-lysyl hydroxylase activity in vitro. Skin Pharmacol 1996; 9: 177–183.
20. Courchay G, Boyera N, Bernard BA, Mahé Y. Messenger RNA expression of steroidogenesis enzymes subtypes in the human pilosebaceous unit. Skin Pharmacol 1996; 9: 169–176.
21. Törmä H, Rollman O, Vahlquist A. Detection of mRNA transcripts for retinoic, vitamin D3, and thyroid hormone (c-erb-A) nuclear receptors in human skin using reverse transcription and polymerase chain reaction. Acta Derm Venereol (Stockh) 1993; 73: 102–107.
22. Harmon CS, Nevins TD. Biphasic effect of 1,25-dihydroxyvitamin D3 on human hair follicle growth and hair fiber production in whole-organ cultures. J Invest Dermatol 1994; 103: 318–322.
23. Elder JT, Fisher GJ, Zhang KY, Eisen D, Krust A, Kastner P, et al. Retinoic acid receptor genes expression in human skin. J Invest Dermatol 1991; 96: 425–433.
24. Fisher GJ, Talwar HS, Xiao JH, Datta SC, Reddy AP, Gaub MP, et al. Immunological identification and functional quantitation of retinoic acid and retinoid X receptor proteins in human skin. J Biol Chem 1994; 269: 20629–20635.
25. Berth-Jones J, Shuttleworth D, Hutchinson PE. A study of etretinate alopecia. Br J Dermatol 1990; 122: 751–755.
26. Berth-Jones J, Hutchinson PE. Novel cycle changes in scalp hair are caused by etretinate therapy. Br J Dermatol 1995; 132: 367–375.
27. Graham RM, James MP, Ferguson DJP, Guerrier CW. Acquired kinking of the hair associated with etretinate therapy. Clin Exp Dermatol 1985; 10: 426–431.
28. Nanda A, Alsaleh QA. Hair discoloration caused by etretinate. Dermatology 1994; 188: 172.
29. Harant H, Lindley I, Uthman A, Ballaun C, Krupitza G, Grunt T, et al. Regulation of interleukin-8 gene expression by all-trans retinoic acid. Biochem Biophys Res Comm 1995; 210: 898–906.
30. Harmon CS, Nevins TD. IL-1α inhibits human hair follicle growth and hair fiber production in whole-organ culture. Lymphokine Cytokine Res 1993; 12: 197–202.
31. Philpott MP, Sanders D, Kealey T. Cultured human hair follicles and growth factors. J Invest Dermatol 1995; 104: 44S-45S.
32. Williams D, Siock P, Stenn K. 13-cis-retinoic acid affects sheath-shaft interactions of equine hair follicles in vitro. J Invest Dermatol 1996; 106: 356–361.
33. Carlberg C. The concept of multiple vitamin D signaling pathways. J Invest Dermatol 1996; 1: 10–14.
34. Carlberg C. The vitamin D3 receptor in the context of the nuclear receptor superfamily. Endocrine 1996; 4: 91–105.
35. Macdonald PN, Dowd DR, Nakajima S, Galligan MA, Reeder MC, Haussler CA, et al. Retinoid X receptors stimulate and 9-cis retinoic acid inhibits 1,25 dihydroxyvitamin D3-activated expression of the rat osteocalcin gene. Mol Cell Biol 1993; 13: 5907–5917.

Fig. 2. Effect of agonists of the non-steroid nuclear receptor superfamily on the growth of dissected human hair follicles in culture in vitro (one representative experiment). Values are expressed as cumulative growth of human hair follicles from day 0; mean $\pm$ SEM ($n=12$ at day 0). (A) Inhibitory effects of all-*trans* RA (10^{-9} M) on the survival of human hair follicles in vitro ($p=0.003$ versus control). (B) Inhibitory effects of the RAR pan-agonist CD367 (10^{-8} M) on the survival of human hair follicles in vitro ($p=0.0001$ versus control). (C) Confirmation (see ref. 22) of the stimulatory effect of vitamin D3 (10^{-8} M) on the growth of human hair follicles in culture in vitro (positive control) ($p=0.001$ versus control). (D) Stimulatory effect of the synthetic RXR agonist CD2425 (10^{-8} M) on the growth rate ($p=0.003$ versus control) and the survival of human hair follicles in culture in vitro. Note that after 20 days, while the hair bulb was intact and the hair follicle was still growing, one could not indicate the length increase due to the curling of the overgrowing follicles in the culture well.