Journal of Cellular Biochemistry 49:46–52 (1992)

Vitamin D Analogues in the Treatment of Psoriasis

Knud Kragballe

Department of Dermatology, Marselisborg Hospital, University of Aarhus, 8000 Aarhus, Denmark

Abstract Psoriasis is a chronic hyperproliferative skin disease in which inflammatory and immunologic processes may play important pathophysiologic roles. Recently the skin has been identified as a target tissue for vitamin D. Because 1,25-dihydroxy vitamin D_3 inhibits epidermal proliferation and promotes epidermal differentiation, it has been introduced for the treatment of psoriasis vulgaris. In addition to 1,25-$(OH)_2$-D_3, synthetic vitamin D_3 analogues have undergone clinical evaluation. Calcipotriol (INN) (calcipotriene [USAN]) has been studied most extensively. Compared with 1,25-$(OH)_2$-D_3, calcipotriol is about 200 times less potent in its effects on calcium metabolism, although similar in receptor affinity. Topical calcipotriol 50 μg/g applied twice daily is efficacious and safe for the treatment of psoriasis. Because topical calcipotriol is slightly more efficacious than betamethasone 17-valerate and dithranol, calcipotriol should be considered a first line drug in the management of psoriasis. These results illustrate that it is possible to separate the vitamin D effects on the cellular level from those on calcium metabolism not only in vitro, but also in a clinical setting.

Key words: calcium metabolism, 1,25-$(OH)_2$-D_3, 1-alpha-OH-D_3, 1,24-$(OH)_2$-D_3, calcipotriol, calcipotriene, epidermal keratinocyte, psoriasis

Psoriasis is a common, chronic skin disorder. The disease appears in several clinical forms, but chronic plaque psoriasis (psoriasis vulgaris) is by far the most common. Clinically psoriasis is characterized by thickened erythematous, well-demarcated areas of skin covered by silvery scales. Microscopic examination of involved skin shows hyperproliferation of epidermal keratinocytes; accumulation of leukocytes, particularly T lymphocytes, neutrophils, and monocytes; and elongation and increased tortuosity of dermal papillary blood vessels. Keratinocyte hyperproliferation is one of the hallmarks of psoriasis. Mitoses are more frequent in psoriatic than in normal epidermis, and keratinocyte turnover is about ten times more rapid. Concomitant with hyperproliferation is incomplete terminal differentiation, shown histologically by parakeratosis and loss of the granular layer. Biochemically, keratin expression is altered, and involucrin and membrane-bound transglutaminase appear prematurely in psoriatic epidermis.

The inflammatory changes seen in psoriasis *may be linked* with the aberrant keratinocyte growth via at least 2 mediator systems. Interleukin-8, detectable in biologically active concentrations in psoriatic lesions, is a potent keratinocyte mitogen. These properties are shared with another potent chemoattractant, the eicosanoid leukotriene B_4. Although keratinocytes by themselves may produce interleukin-8 and leukotriene B_4, the infiltrating leukocytes are probably the major sources. Recently the interaction between T lymphocytes and keratinocytes has attracted much interest. The T lymphocytes, predominantly helper memory cells (CD4+), are activated as shown by their expression of HLA-DR, IL-2 receptors, and Ki-67 antigen. Interferon-gamma (IFN-gamma), an activated T-cell derived cytokine, may be a mediator of many of the changes seen in psoriasis.

VITAMIN D METABOLISM AND RECEPTORS IN NORMAL SKIN

The skin is responsible for producing vitamin D_3 from 7-dehydrocholesterol on exposure to sunlight. After successive hydroxylations in the liver and in the kidney, 1,25-dihydroxy-vitamin D_3, the bioactive form of vitamin D_3 is formed. Recently, cultured neonatal human keratinocytes were also found to produce 1,25-$(OH)_2$-D_3 from 25-OH-D_3, indicating that human skin

Received January 23, 1992; accepted January 24, 1992.

Address reprint requests to Dr. Knud Kragballe, Department of Dermatology, Marselisborg Hospital, University of Aarhus, 8000 Aaurhus, Denmark.

might be an alternative source of this metabolite [1].

The skin contains a specific receptor for 1,25-$(OH)_2$-D_3 (VDR). The presence of this receptor has been examined in human skin [2,3] and in cultures of human epidermal keratinocytes [4] and human dermal fibroblasts [4,5]. In normal skin VDR antigens are expressed in keratinocytes of all epidermal layers (except those of stratum corneum) and in cells of epidermal appendages [6]. Furthermore, 50–75% of Langerhans cells, macrophages, and T lymphocytes express VDR [6].

These findings strongly support the hypothesis that the epidermal keratinocytes and the skin immune system may be targets for 1,25-$(OH)_2$-D_3. The effects on epidermal keratinocyte proliferation and differentiation have been studied most thoroughly. At physiologic concentrations 1,25-$(OH)_2$-D_3 causes a decrease in proliferation and an increase in the morphologic and biochemical differentiation of cultured keratinocytes [7,8]. The mechanisms by which 1,25-$(OH)_2$-D_3 cause inhibitory effects on keratinocytes proliferation are not understood. It may act by decreasing cell sensitivity to growth factors acting via the EGF receptor, which at least in breast cancer cell lines are decreased by 1,25-$(OH)_2$-D_3 [9]. The effects of growth factors may be affected by other mechanisms. Thus, 1,25-$(OH)_2$-D_3 enhances the antiproliferative effect and transcription of TGF-beta-1 in human keratinocyte cultures [10]. A change in the expression of proto-oncogenes may also be involved. In human keratinocyte cultures 1,25-$(OH)_2$-D_3 causes a decrease of the c-myc mRNA levels [13], an effect that occurs in parallel with the inhibition of DNA synthesis. Until the role of the c-myc protein in the growth keratinocytes is exactly known, the importance of this finding remains unknown. The 1,25-$(OH)_2$-D_3 effects described above involve gene transcription. In addition 1,25-$(OH)_2$-D_3 can induce keratinocyte differentiation by a rapid increase of intracellular calcium levels [11,12].

The emerging concept of the skin as an immune competent tissue makes it relevant to assess the effects of 1,25-$(OH)_2$-D_3 on the skin immune system. In the human allogeneic mixed epidermal cell lymphocyte reaction (MECLR), 1,25-$(OH)_2$ causes an inhibition, which is maximal at a concentration of 10^{-8} M [14]. In experiments designed to assess the effects of 1,25-$(OH)_2$ on the 2 cell populations involved in this reaction, 1,25-$(OH)_2$-D_3 was found to affect preferentially the epidermal cells. The potential relevance of these results is supported by studies of skin allograft survival. In mice treated with oral 1-alpha-OH-D_3, a prodrug of 1,25-$(OH)_2$-D_3, skin allograft survival was significantly prolonged [15]. In addition to interference with allogeneic cell activation, 1,25-$(OH)_2$-D_3 may inhibit IL-1 induced cell activation. Thus, the expression of IL-8 mRNA as well as the production of IL-8 by IL-1 stimulated cultured human keratinocytes, fibroblasts and monocytes are inhibited by 1,25-$(OH)_2$-D_3 in a dose-dependent manner [16]. This finding indicates that 1,25-$(OH)_2$-D_3 may interact with and regulate a cytokine pathway responsible for the accumulation of leukocytes during skin inflammation.

In conclusion, it is becoming apparent that 1,25-$(OH)_2$-D_3, apart from regulation of the growth of skin cells, may have profound effects on the immunologic and inflammatory processes taking place in the skin.

VITAMIN D METABOLISM IN PSORIASIS

There exist conflicting results regarding the vitamin D metabolism in psoriatic patients. Morimoto et al. [17] observed no difference in the mean levels of circulating 1,25-$(OH)_2$-D_3 between psoriatics and normal subjects, whereas Staberg et al. [18] reported reduced serum 1,25-$(OH)_2$-D_3 concentrations in psoriatics with disseminated disease. More recently Smith et al. [19] and Guilhou et al. [20] found normal serum 1,25-$(OH)_2$-D_3 levels in patients with moderate to extensive psoriasis. An inverse relationship between the severity of psoriasis and the serum 1,25-$(OH)_2$-D_3 level [21] may probably explain these conflicting results. It is unknown whether the slightly decreased levels of circulating 1,25-$(OH)_2$-D_3 found in some psoriatics with severe disease is due to decreased production or increased degradation, either systemically or locally in the skin.

Furthermore, severe psoriasis has been observed in association with hypoparathyroidism [22] and hypocalcemia [23]. In these patients the psoriasis improved when the serum calcium was restored. Although these case reports demonstrate that fluctuations in serum calcium can precipitate psoriasis, it is important to realize that the reported patients had normal levels of 1,25-$(OH)_2$-D_3 and that all parameters of calcium and bone metabolism are normal in larger groups of patients [20,24]. Taken together the

available data do not support the idea that psoriasis is a manifestation of abnormal vitamin D or calcium metabolism.

A related question is whether psoriatic skin is less sensitive to 1,25-$(OH)_2$-D_3 than normal skin. It has been reported that cultures of dermal fibroblasts and epidermal keratinocytes from psoriatics are partially resistant to the antiproliferative effect 1,25-$(OH)_2$-D_3 despite normal binding to the VDR [25,26]. However, one of these investigators was unable to repeat these results [19]. Because other investigators have failed to show a difference between psoriatic fibroblasts and normal fibroblasts, there is apparently no inherent insensitivity of psoriatic fibroblasts to the actions of 1,25-$(OH)_2$-D_3.

Immunochemical methods have been applied to investigate the in situ expression of VDR in psoriatic skin. Compared to normal skin, nonlesional psoriatic skin reveals a nearly identical staining pattern [6]. In contrast, lesional psoriatic skin exhibits a significant increase of VDR expression both in basal and suprabasal layers and shows a remarkable change of the immune cell pattern: the density and proportion of VDR positive T lymphocytes and macrophages are higher in the epidermis and in the perivascular papillary dermis [6]. If the immunoreactive epitope on VDR reflects the hormone-binding capacity of psoriatic skin, then psoriasis should be a disease sensitive to 1,25-$(OH)_2$-D_3.

TREATMENT WITH VITAMIN D_3 ANALOGUES IN PSORIASIS

Oral 1-Alpha-Hydroxyvitamin D_3 (1-Alpha-OH-D_3)

Although the rationale for using vitamin D_3 analogues in psoriasis is their ability to reverse epidermal hyperproliferation and to promote epidermal differentiation, it is important to realize that it was a chance observation that stimulated the interest for vitamin D_3 in psoriasis. A patient received oral 1-alpha-OH-D_3 0.75 μg/day for senile osteoposis [27]. After 2½ months the psoriatic skin lesions were dramatically improved. Later the same group of investigators reported improvement in 13 out of 17 psoriatics treated with oral 1-alpha-OH-D_3 1.0 μg/day for 6 months [28]. In another open study 10 out of 15 patients improved during treatment with oral 1-alpha-OH-D_3 1 μg/day for 4–6 months [29]. No adverse events or serum calcium changes were reported in these trials with 1-alpha-$(OH)_2$-D_3.

Until controlled trials with a large number of patients have been conducted, it is impossible to make any conclusions about the efficacy and safety of oral 1-alpha-OH-D_3 in psoriasis. Because 1-alpha-OH-D_3 is a prodrug of 1,25-$(OH)_2$-D_3, it must be anticipated that the safety problems will be similar to those with oral 1,25-$(OH)_2$-D_3, which are described below.

Oral 1,25-Dihydroxy-Vitamin D_3

As for 1-alpha-OH-D_3, the anti-psoriatic effect of oral 1,25-$(OH)_2$-D_3 has only been assessed in open studies without controls [17,19,29]. Approximately half of the treated patients have shown some degree of improvement. It should, however, be stressed that various doses (0.5–2.0 μg/day) were used in these studies, all of which included only a low number of patients. Even from this limited experience there appears to be a problem with the safety of oral 1,25-$(OH)_2$-D_3. In a study in which the dosage was increased by 0.25 to 0.5 μg every 2 weeks, hypercalciuria was usually observed, when the divided daytime dosage exceeded 0.75 μg/day. Although a change to administer 1,25-$(OH)_2$-D_3 as a single dose at bedtime reduced the incidence of hypercalciuria, 2 out of 14 patients had to be withdrawn because of persistent hypercalciuria [19].

It must, therefore, be concluded that the therapeutic index of oral 1,25-$(OH)_2$-D_3 is low, and that a safe and effective dose has not been established.

Topical 1,25-Dihydroxy-Vitamin D_3

Because of the problems with orally administered 1,25-$(OH)_2$-D_3 , many investigators have turned to topical application [17,19,29–34]. Topical 1,25-$(OH)_2$-D_3 has produced mixed results. In the initial open studies an improvement was observed. However, in the double-blind studies no benefit was found compared to placebo. Although the usage of different doses and different vehicles makes it difficult to make direct comparisons between these studies, it appears that doses of 3 μg/g or above are effective when formulated in a petrolatum based ointment. It is, however, questionable whether such doses are safe. In one study 1,25-$(OH)_2$-D_3 was applied twice daily at a concentration of 15 μg/g without producing hypercalcemia or hypercalciuria [32]. In contrast, a comparison between the concentrations 3 μg/g and 15 μg/g showed the higher concentra-

tion to cause hypercalciuria when the treated skin area exceeded 600–1,200 cm^2 [33].

It can, therefore, be concluded that the window between efficacy and side effects is quite narrow for topical 1,25-$(OH)_2$-D_3. This intrinsic problem may limit the clinical use of topical 1,25-$(OH)_2$-D_3.

Topical Calcipotriol (Calcipotriene)

Because of the potent effects of 1,25-$(OH)_2$-D_3 on calcium metabolism, systemic absorption of even small amounts from the skin may affect calcium metabolism. This is the reason why it is difficult to identify an effective and at the same time safe dose for psoriasis. Therefore, new vitamin D analogues with potent cell regulating properties, but lower risk of inducing calcium-related side effects, have been synthesized. Calcipotriol (MC 903) is a synthetic 1,24-dihydroxy-vitamin D analogue containing a double bond and a cyclopropane ring in the side chain [35] (Fig. 1). Calcipotriol (INN) is the generic name designated by WHO. In USA the generic name is calcipotriene (USAN). In the following the name calcipotriol will be used.

The modification of the side chain results in a rapid transformation into inactive metabolites when given i.v. to rats. As a consequence of these pharmacokinetic properties, calcipotriol is about 200 times less potent than 1,25-$(OH)_2$-D_3 in producing hypercalcemia and hypercalciuria after oral administration in rats [36]. In contrast, calcipotriol and 1,25-$(OH)_2$-D_3 are equipotent in their affinity for the VDR and in their effects on keratinocyte growth in vitro. This unique pharmacologic profile of calcipotriol makes it an interesting candidate for the topical treatment of psoriasis. Furthermore, less than 1% of calcipotriol is absorbed systemically after a single application to a psoriatic skin lesion.

In double-blind, placebo-controlled studies calcipotriol cream or ointment have been shown to improve psoriasis [37–39]. Maximum improvement is observed at concentration of 50 μg/g. When applied twice daily the improvement is detectable within 1–2 weeks and maximal at 6–8 weeks. Most patients will experience a marked improvement, although a complete clearance is only obtained in a minority.

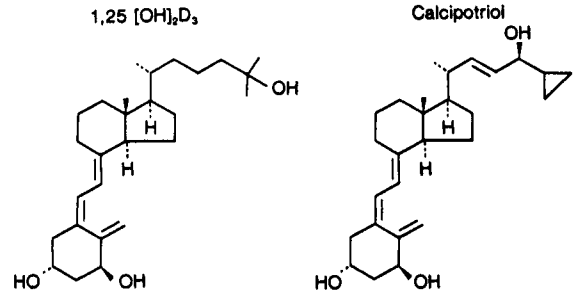

Fig. 1. Structural formulas of 1,25-$(OH)_2$-D_3 and calcipotriol.

In multicenter studies conducted in several European countries and in Canada, calcipotriol ointment 50 μg/g has been compared with betamethasone 17-valerate ointment 0.1% and dithranol cream, two drugs widely used in the management of psoriasis. Calcipotriol was compared with betamethasone 17-valerate in both a right-left comparison (n = 35) [40] and a parallel group comparison (n = 409) [41]. Both treatments caused a marked improvement, but calcipotriol was slightly more effective than betamethasone 17-valerate. Similar results were found when calcipotriol was compared with dithranol (unpublished). In this study increasing concentrations (0.1%–2%) of dithranol was applied once daily in the so called "short contact mode."

The potential effect on systemic calcium metabolism is the principal dose-limiting factor in the use of calcipotriol and other vitamin D analogues. In the studies with calcipotriol, patients were provided with a maximum of 100 g ointment per week. This amount is sufficient for treating approximately 20% of the body surface area twice daily. In more than 2000 psoriatic patients treated in this way, there has been detected no change of serum calcium levels. However, there are 2 cases of hypercalcemia developed after application of 400 g ointment in 10 days [41] and of 200 g ointment in 7 days [42]. Although the serum calcium normalized in both cases a few days after stopping calcipotriol treatment, these reports illustrate that excessive use of calcipotriol ointment may result in transcutaneous absorption of calcipotriol in quantities sufficient to influence calcium metabolism. Therefore, it becomes important to determine whether treatment with calcipotriol ointment 50 μg/g in the recommended doses (maximum of 100 g per week) may affect calcium metabolism, even if serum calcium remains unchanged. This question has been addressed in a double-blind, placebo-controlled study. Patients were put on a calcium-energy-fixed diet and treated for 3 weeks. Compared with the placebo-treated patients, patients

treated with calcipotriol did not show a change in any of the parameters of calcium/bone metabolism, which included the serum levels of ionized calcium, phosphorous, calcitonin, osteocalcin, parathyroid hormone, bone-related alkaline phosphatase, and 24 h urine hydroxyproline, phosphorous, and calcium [24]. A related question has been whether a synthetic vitamin D analogue might decrease the formation of the natural form of the hormone. To the extent that the synthetic analogue and 1,25-$(OH)_2$-D_3 are different in their biological activities, this might result in a 1,25-$(OH)_2$-D_3 deficiency, systemically or locally in the skin. In the study described above [24], treatment with calcipotriol did not change the serum levels of 1,25-$(OH)_2$-D_3 or 25-OH-D_3. These results clearly demonstrate that patients with psoriasis can be safely treated with topical calcipotriol.

A skin irritation is the only clinically relevant side effect observed during calcipotriol treatment. In most cases this irritant reaction is mild and may disappear during continued treatment. The face and skin folds are particularly sensitive to calcipotriol ointment. Therefore, calcipotriol should not be used in the face and only cautiously in the skin folds.

Unfortunately there is a gradual recurrence of psoriasis upon stopping treatment with calcipotriol. Because many patients may require maintenance therapy, it becomes important to assess the long term efficacy and safety of calcipotriol treatment. In patients receiving calcipotriol therapy daily for about 6 months we have found that the initial improvement can be maintained in most of the patients and that serum calcium remains normal [43]. Furthermore, the type, severity, and incidence of side effects were similar to those seen in short-term studies. In another study, in which patients were treated as required over the course of 1 year, calcipotriol ointment also provided effective and safe control of psoriasis (unpublished).

It can be concluded that calcipotriol ointment 50 μg/g is an effective and safe drug for the topical treatment of mild to moderate psoriasis. If used according to the guidelines described above, it should be considered a first line drug for the management of psoriasis. Calcipotriol is already available in some European countries and is being developed for the North American market.

1,24-Dihydroxy-Vitamin D_3

1,24-$(OH)_2$-D_3 is another synthetic vitamin D_3 analogue developed for topical use in psoriasis. 1,24-$(OH)_2$-D_3 is equipotent with 1,25-$(OH)_2$-D_3 in its affinity for the VDR and in its effects on keratinocyte growth in vitro [44,45]. Although less hypercalcemia is induced after a single intravenous dose of 1,24-$(OH)_2$-D_3 in rats, the doses inducing hypercalcemia is similar for 1,24-$(OH)_2$-D_3 and 1,25-$(OH)_2$-D_3 [45]. These results indicate that 1,24-$(OH)_2$-D_3 may be advantageous over 1,25-$(OH)_2$-D_3 in psoriasis, but much less selective in its pharmacodynamic actions than calcipotriol.

Unfortunately the clinical experience with 1,24-$(OH)_2$-D_3 is rather limited [46,47]. In an open study 1,24-$(OH)_2$-D_3 ointment 1–4 μg/g was applied under occlusion once daily in 7 psoriatic patients [46]. Only small skin lesions were treated. Within one month 1,24-$(OH)_2$-D_3 ointment produced improvement, irrespective of the concentration used. The degree of improvement was similar to that observed with betamethasone 17-valerate ointment 0.1%. No change of serum calcium was found in these patients. Because 1,24-$(OH)_2$-D_3 was only applied to limited skin areas the efficacy to safety ratio for 1,24-$(OH)_2$-D_3 remains to be evaluated.

MODE OF ACTION IN PSORIASIS

The mode of action of vitamin D analogues in psoriasis is not completely understood. The rationale for their use is their ability to reverse the epidermal hyperproliferation and to promote the epidermal differentiation. Indeed the various epidermal keratins, which are markers of proliferation and differentiation, return to normal during calcipotriol treatment [48,49]. These results are, however, similar to those observed with other anti-psoriatic therapies, such as corticosteroids, dithranol and PUVA.

Because vitamin D_3 analogues possess immunosuppressive effects, investigators have addressed the question whether their anti-psoriatic effect may be attributed to their immunomodulating properties. This hypothesis has gained additional interest in light of the dramatic response of psoriasis to cyclosporin A, a drug which inhibits T-cell activation and cytokine release. When markers of leukocyte subpopulations and epidermal growth are assessed simultaneously

during calcipotriol treatment, the influence on lymphocytes is rather limited and not as pronounced as for betamethasone 17-valerate [50,51]. Thus, there is little immunohistochemical evidence to support an important immunosuppressive role of this vitamin D_3 analogue in psoriasis.

A better understanding of the mechanisms of action of vitamin D_3 analogues in psoriasis is of theoretical as well as practical importance. A knowledge about the mode of action may enable investigators to screen new vitamin D analogues with more potent and selective activities, and thereby optimize the antipsoriatic effect. The vitamin D analogues applied so far in psoriasis are characterized by a relatively strong antiproliferative effect. The availability of novel analogues with an extremely strong immunosuppressive effect [52] may enable investigators to test whether such analogues are more effective in psoriasis. Another fundamental question is whether the variable clinical response to vitamin D analogues in psoriasis reflects variable disease activity or variable sensitivity of the skin vitamin D system. When these questions have been answered, we may be able to offer an optimal therapy with vitamin D analogues to psoriatic patients.

REFERENCES

1. Bikle DD, Nemanic MK, Gee E, Elias P: J Clin Invest 78:557–566, 1986.
2. Stumpf WE, Sar M, Reid FA, Tanaka Y, De Luca HF: Science 206:1188–1190, 1979.
3. Feldman D, Chen T, Hirst M, Colston K, Karasek M, Cone C: J Clin Endocrinol Metabol 51:1463–1465, 1980.
4. Clemens TL, Adams JS, Horiuchi N, Gilchrest BA, Cho H, Tsuchiya Y, Matsuo N, Suda T, Holick MF: J Clin Endocrinol Metabol 56:824–830, 1983.
5. Eil C, Marx SJ: Proc Natl Acad Sci USA 78:2562–2566, 1981.
6. Milde P, Hauser U, Simon T, Mall G, Ernst V, Haussler MR, Frosch P, Rauterberg EW: J Invest Dermatol 97: 230–239, 1991.
7. Hosomi J, Hosoi J, Abe E, Suda T, Kuroki T: Endocrinology 113:1950–1957, 1983.
8. Smith EL, Walworth NC, Holick MF: J Invest Dermatol 86:709–714, 1986.
9. Koga M, Eisman JA, Sutherland RL: Cancer Res 48: 2734–2739, 1988.
10. McLane JA, Kim H-J, Abdelkader N, Katz M: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p 264.
11. Yada Y, Ozeki T, Meguro S, et al: Biochem Biophys Res Commun 163:1517–1522, 1989.
12. Tang W, Ziboh VA: J Invest Dermatol 96:134–138, 1991.
13. Matsumoto K, Hashimoto K, Nishida Y, Hashiro M, Yoshikawa K: Biochem Biophys Res Commun 166:916–923, 1990.
14. Bagot M, Charue D, Pamphile R, Revuz J: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p 87.
15. Veyron P, Pamphile R, Binderup L, Touraine JL: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p 196.
16. Larsen CG, Kristensen M, Paludan K, Deleuran B, Thomsen MK, Zachariae C, Kragballe K, Matsushima K, Thestrup-Pedersen K: Biochem Biophys Res Commun 176:1020–1026, 1991.
17. Morimoto S, Yoshikawa K, Kozuka T, Kitano Y, Imanaka S, Fukuo K, Koh E, Kumahara Y: Br J Dermatol 115: 421–429, 1986.
18. Staberg B, Oxholm A, Klemp P, *Christansen C: Acta* Dermatol Venereol (Stockh) 67:65–68, 1987.
19. Smith EL, Pincus SH, Donovan L, Holick MF: J Am Acad Dermatol 19:360–364, 1988.
20. Guilhou JJ, Colette C, Monpoint S, Lancrenon E, Guillot B, Monnier L: Acta Derm Venereol (Stockh) 70:351–354, 1990.
21. Morimoto S, Yoshikawa K, Fukuo K, Shiraishi T, Koh E, Imanaka S, Kitano S, Ogihara T: J Dermatol Sci 1:277–282, 1990.
22. Risum G: Br J Dermatol 89:309–312, 1973.
23. Stewart AF, Battaglini-Sabetta J, Millstone L: Ann Intern Med 100:677–680, 1984.
24. Mortensen L, Kragballe K, Schifter S, Charles P: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p. 249.
25. MacLaughlin JA, Gange W, Taylor D, Smith EL, Holick MF: Proc Natl Acad Sci USA 82:5409–5412, 1985.
26. Abe J, Kondo S, Nishii Y, Kuroki T: J Clin Endocrinol Metab 68:851–854, 1989.
27. Morimoto S, Kumahara Y: Med J Osaka Univ 35:51–54, 1985.
28. Holland DB, Wood EJ, Roberts SG, West MR, Cunliffe WJ: Skin Pharmacol 2:68–76, 1989.
29. Morimoto S, Yoshikawa K: Arch Dermatol 125:231–234, 1989.
30. Van De Kerkhof PCM, Van Bokhoven M, Zultak M, Czarnetzki BM: Br J Dermatol 120:661–664, 1989.
31. Henderson CA, Papworth-Smith J, Cunliffe WJ, Highet AS, Shamy HK, Czarnetzki BM: Br J Dermatol 121:446, 1989.
32. Holick MF, Pochi P, Bhawan J: J Invest Dermatol 92:446, 1989.
33. Langner A, Verjans H, Stapor V, Mol M, Elzerman: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p 58.
34. Zultak M: Fifth International Psoriasis Symposium, San Francisco, USA, July 10–14, 1991, abstract p 108.
35. Calverley MJ: Tetrahedron 43:4609–4619, 1988.
36. Binderup L, Bramm E: Biochem Pharmacol 37:889–895, 1988.
37. Kragballe K, Beck H-I, Søgaard H: Br J Dermatol 119: 223–230, 1988.
38. Staberg B, Roed-Pedersen J, Menné T: Acta Dermatol Venereol (Stockh) 69:147–150, 1989.
39. Kragballe K: Arch Dermatol 125:1647–1652, 1989.

40. Kragballe K, Gjertsen BT, De Hoop D, Karlsmark T, Van De Kerkhof P, Larkö P, Niesboer C, Roed-Petersen J, Strand A, Tikjøb G: Lancet 337:193–196, 1991.
41. Cunliffe WJ, Claudy A, Fairiss G, Goldin D, Gratton D, Holden CA, Berth-Jones J, Maddin WS, Ortonne J-P, Henderson CA, Young M: J Am Acad Dermatol, in press.
42. Dwyer C, Chapman RS: Lancet 338:764–765, 1991.
43. Kragballe K, Fogh K: Acta Dermatol Venereol (Stockh), 71:475–478, 1991.
44. Matsumoto K, Hashimoto K, Kiyoki M, Yamamoto M, Yoshikawa K: J Dermatol 17:97–103, 1990.
45. Matsunaga T, Yamamoto M, Mimura H, Ohta T, Kiyoki M, Ohba T, Naruchi T, Hosoi J, Kuroki: J Dermatol *17:135–142*, 1990.
46. Kato T, Rokugo M, Terui T, Tagmai H: Br J Dermatol 115:431–433, 1986.
47. Tagami H, Kato T, Terui T, Tadaki T: Proc seventh Workshop on Vitamin D, California, USA, April 1988, pp 958–967.
48. Holland DB, Roberts SG, Russell A, Wood EJ, Cunliffe WJ: Br J Dermatol 122:284, 1990.
49. De More S, De Jong EGJM, Van De Kerkhof PCM: Br J Dermatol 123:291–295, 1990.
50. De Jong EMGJ, Van De Kerkhof PCM: Br J Dermatol 124:221–229, 1991.
51. Nieboer C, Verburgh CA: Invest Dermatol 93:210, 1989.
52. Binderup L: Eighth Workshop on Vitamin D, Paris, France, July 5–10, 1991, abstract p 194.