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1α,25-DIHYDROXYVITAMIN D_3 INHIBITS PHORBOL ESTER-DEPENDENT CHEMICAL CARCINOGENESIS IN MOUSE SKIN

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Summary: The effect of topical application of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) on the promotional phase of skin tumor formation in mice was evaluated using 7,12-dimethylbenz[a]anthracene as the tumor initiator and 12-O-tetradecanoylphorbol-13-acetate (TPA) as the tumor promoter. Fifteen weeks of twice weekly topical application of 1α,25-$(OH)_2D_3$ 1 hour prior to topical treatment with 16 nmol of TPA inhibited tumor formation in a dose-dependent manner. Doses of 0.25-0.50 nmol of the vitamin D_3 metabolite inhibited tumor formation approximately 50% and had no significant effect on the survival or weight gain of the mice. These results indicate that in addition to maintaining calcium homeostasis and affecting the growth and differentiation of certain neoplastic cells, 1α,25-$(OH)_2D_3$ can also suppress the formation of chemically induced tumors.

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$), the hormonally active metabolite of vitamin D_3, is formed by the sequential 25- and 1α-hydroxylation of vitamin D_3 in the liver and kidney, respectively. 1α,25-$(OH)_2D_3$ regulates calcium and phosphate transport in the intestine and mineral mobilization in the bone by mechanisms involving its interaction with specific, high affinity, low capacity, protein receptors (1,2).

Recent studies have demonstrated that a number of cancerous cells and cell lines also possess 1α,25-$(OH)_2D_3$ receptors. Addition of nM concentrations of the metabolite to cultured Hs695T human melanoma cells or T47D human breast cancer cells causes a marked suppression in replication (3,4), while inclusion of 1α,25-$(OH)_2D_3$ in the culture medium causes both M1 murine and HL60 human myeloid leukemia cell lines to slow their rate of replication and to differen-

ABBREVIATIONS

1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; TPA, 12-O-tetradecanoylphorbol-13-acetate; DMBA, 7,12-dimethylbenz[a]anthracene.

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tiate into macrophage-like cells (5,6). In vivo, 1α,25-$(OH)_2D_3$ can significantly prolong the survival time of mice innoculated with the M1 leukemia cells (7). Furthermore, a preliminary investigation (8) suggests that 1α-hydroxyvitamin D_3, a metabolic precursor of 1α,25-$(OH)_2D_3$, can reduce the size of transplanted sarcoma 180 cells in mice, and reduce the number of lung metastases resulting from implantation of Lewis lung adenoma cells in mice. Thus, in addition to its well known effects on calcium homeostasis, 1α,25-$(OH)_2D_3$ appears to have a chemotherapeutic potential of modulating the growth of tumor cells. The ability of 1α,25-$(OH)_2D_3$ to suppress the proliferation and induce the differentiation of preexistent neoplastic cells suggested a need for experiments to determine the effect of the active metabolite of vitamin D_3 on the induction of tumors in a chemical carcinogenesis model.

Experimentally, one of the most widely used systems to study the sequential development of cancer is the initiation-promotion model on mouse skin (9,10). In this model, a low subcarcinogenic dose of a complete carcinogen is applied once to the skin of mice to initiate the carcinogenic process. After approximately a week, this treatment is followed by repeated applications of a tumor promoter, a noncarcinogenic chemical that causes the production of tumors in skin treated with the initiator. The polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene (DMBA), is the most commonly used tumor initiator, and the macrocyclic diterpene phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA), is the most widely studied tumor promoter.

The fact that TPA can modulate cellular differentiation (11,12) coupled with recent findings that cells treated with TPA exhibit rapid changes in calcium flux (13-15) and undergo stimulation of calcium dependent processes, such as phospholipid turnover (16), indicated that an evaluation of the effect of 1α,25-$(OH)_2D_3$ in the initiation-promotion tumor model was warranted.

MATERIALS & METHODS

Chemicals. 1α,25-$(OH)_2D_3$ was synthesized as previously described (17) and the analytically pure compound was dissolved in spectral grade acetone and stored at -90° between uses. DMBA was obtained from Aldrich Chemical Co., Milwaukee, WI and TPA was purchased from Dr. Peter Borchert, Chemical Carcinogenesis Inc.,

Eden Prairie, MN. Spectral grade acetone was purchased from Burdick and Jackson, Muskegon, MI.

Animals. Female CD-1 mice (6-7 weeks old) were purchased from Charles River Breeding Laboratories, North Wilmington, MA. Thirty mice in each treatment group (15 mice/cage) were shaved on the dorsal surface with surgical clippers 2 days before treatment with a single topical dose of 20 nmol of DMBA in 200 µl of acetone. . Seven days later the animals started receiving twice weekly topical treatment with 1α,25-$(OH)_2D_3$ in 200 µl of acetone. One hour after treatment with the vitamin D_3 metabolite, the mice were treated topically with 16 nmol of TPA in 200 µl of acetone. All manipulations of the chemicals and all chemical treatments of the mice were performed under subdued light. Weight gain of the mice was monitored during the course of the experiments. Skin tumors of at least 2 mm in diameter were recorded at 9, 12 and 15 weeks of promotion. Differences between experimental groups in the percent of tumor bearing animals and tumor multiplicity were analyzed statistically by the fourfold contingency test of Mainland and Murray (18) and Student's t test, respectively.

RESULTS

Table 1 summarizes the survival, total body weight and tumorigenicity data obtained from two separate experiments in which DMBA-initiated mice were treated topically with solvent or various amounts of 1α,25-$(OH)_2D_3$ 1 hr prior to each topical application of 16 nmol of TPA. In the first experiment, half of the solvent treated control animals had tumors by 9 weeks of promotion with TPA and almost 90% of the animals were bearing one or more tumors by 15 weeks of promotion. The corresponding tumor multiplicity data at 9 and 15 weeks of promotion was 2.45 and 8.61 tumors/mouse, respectively.

Twice weekly co-administration of 0.5 nmol of 1α,25-$(OH)_2D_3$ with 16 nmol of TPA for 15 wks had no effect on animal survival (97%), and little, if any, effect on total body weight. However, this dose of 1α,25-$(OH)_2D_3$ caused significant ($p < 0.05$) decreases in the percent of animals bearing tumors at 12 weeks (48% inhibition) and 15 weeks (33% inhibition). 1α,25-$(OH)_2D_3$ also decreased the number of tumors per mouse. The 82%, 60% and 54% inhibition in the average number of tumors per mouse observed at 9, 12 and 15 weeks of promotion, respectively, were all statistically significant ($p < 0.05$). The inhibition of tumor formation by the vitamin D_3 metabolite was dose dependent since a four fold higher dose (2.0 nmol/application) was associated with over an 80% inhibition of tumorigenicity at 9, 12 and 15 weeks of treatment. However, this dosage schedule was toxic to the mice since there was a 27% mortality rate and surviving mice weighed 70% of control animals. A 10

TABLE 1

EFFECT OF 1α,25-$(OH)_2D_3$ ON PAPILLOMA FORMATION ON MOUSE SKIN INDUCED BY 7,12-DIMETHYLBENZ[a]ANTHRACENE (DMBA) AND PROMOTED BY 12-TETRADECANOYLPHORBOL-13-ACETATE (TPA)

EXPERIMENT	COMPOUND	NMOL	% SURVIVAL	BODY WEIGHT	% TUMOR BEARING ANIMALS			TUMORS/MOUSE		
			15 wk	15 wk	9 wk	12 wk	15 wk	9 wk	12 wk	15 wk
				(grams)				(Mean ± S.E.)		
	Acetone	-	97	32.5	47	83	88	2.45 ± 0.45	6.98 ± 1.06	8.61 ± 1.09
1	1α,25-$(OH)_2D_3$	0.5	97	30.7	20	43*	59*	0.43 ± 0.22*	2.70 ± 0.72*	3.39 ± 0.91*
		2.0	73	23.0	4*	12*	18*	0.36 ± 0.36*	0.76 ± 0.68*	0.82 ± 0.68*
		10.0	0	-	-	-	-	-	-	-
	Acetone	-	83	32.9	77	96	92	3.77 ± 0.62	7.61 ± 0.88	10.60 ± 1.28
2	1α,25-$(OH)_2D_3$	0.125	97	31.9	37*	70*	83	1.70 ± 0.56*	5.23 ± 1.05	8.21 ± 1.58
		0.250	100	32.1	33*	70*	82	1.57 ± 0.59*	4.50 ± 0.99*	7.61 ± 1.23
		0.50	93	30.6	34*	59*	77	1.34 ± 0.41*	4.14 ± 0.97*	5.87 ± 1.19*

Seven days after a single topical dose of 20 nmol of DMBA, the 30 animals in each treatment group were treated twice weekly with topical applications of acetone (solvent controls) or 1α,25-$(OH)_2D_3$. One hour after each treatment with acetone or 1α,25-$(OH)_2D_3$ the mice were treated topically with 16 nmol of TPA. Values marked with an asterisk (*) were significantly different ($p < 0.05$) from values obtained with acetone treatment (see Materials and Methods).

nmol/application dosage schedule of 1α,25-$(OH)_2D_3$ resulted in an 85% mortality rate after 3 weeks of treatment and no mice survived to the time of the first tumor count at 9 weeks. Control groups of mice that were either initiated with DMBA but not treated with TPA, or that were not initiated with DMBA but were treated with TPA, failed to develop any skin tumors. Survival and body weight data for these mice were comparable to the data summarized in Table 1 for the solvent treated animals.

The second experiment summarized in Table 1 indicates that doses of 1α,25-$(OH)_2D_3$ below 0.5 nmol/application were also effective in reducing both the number of papillomas and the percent of mice with tumors. These low dose effects were most pronounced at the early stages of promotion and by 15 weeks only the 0.5 nmol dose had a statistically significant ($p < 0.05$) effect on tumor multiplicity. Additional control experiments indicated that animals initiated with DMBA and treated twice weekly with 0.5 nmol of 1α,25-$(OH)_2D_3$ in the absence of treatment with TPA did not develop any tumors.

DISCUSSION

The results of the present investigation indicate that 1α,25-$(OH)_2D_3$ can suppress the formation of phorbol ester-induced skin tumors in mice in a dose-dependent manner at concentrations free of apparent toxicity. Previous studies have shown that anti-inflammatory steroids and retinoids are potent inhibitors of mouse skin tumor promotion by TPA (19-21), and several other compounds such as protease inhibitors (22), cyclic nucleotides (22), inhibitors of prostaglandin synthesis (23), difluromethylornithine (24) and diazepam (25) are variously effective at suppressing TPA-mediated tumor formation. Comparison of the anti-tumor activity of 1α,25-$(OH)_2D_3$ with that observed for trans-retinoic acid (data not shown) indicated that the vitamin D_3 metabolite was at least as potent an inhibitor as the retinoid.

The mechanism by which 1α,25-$(OH)_2D_3$ is inhibiting tumor formation is not known. Since as little as 0.125 nmol of 1α,25-$(OH)_2D_3$ can inhibit the tumor promoting activity of a 64-fold molar excess of TPA, a direct chemical interaction between the two compounds appears unlikely. Recently, tumor

promotion has been resolved into discrete stages (26,27). Morphologically, the first step is characterized by the appearence of dark basal keratinocytes after limited application of TPA. The fact that changes in calcium flux are a rapid consequence of the application of TPA to cells (13-15) coupled with the finding that the calcium ionophore A23187 is a first stage promoter (27), suggests a possible role for $1\alpha,25\text{-}(OH)_2D_3$ at this stage of the promotion process. However, the retinoids that inhibit tumor promotion appear to do so by blocking a critical second stage process, which biochemically is characterized by the induction of ornithine decarboxylase and polyamine levels (19,24). Recent studies indicate that calcium-dependent systems such as cyclic-AMP independent protein kinases may be involved in the anti-tumor action of the retinoids (28). Thus it is also possible that $1\alpha,25\text{-}(OH)_2D_3$ is effecting the second stage of promotion. The apparent importance of calcium in both the first and second stages of tumor promotion, and calcium's emerging role in influencing cellular differentiation (29,30) suggests that $1\alpha,25\text{-}(OH)_2D_3$, as the principal physiologic regulator of calcium homoeostasis, may be a useful tool in the understanding of certain aspects of the neoplastic process.

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