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Phytol Is a Novel Tumor Promoter on ICR Mouse Skin

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Phytol is a branched, long-chain aliphatic alcohol which has various biological effects. In this study, we examined phytol as a tumor promoter in a mouse skin initiation-promotion model, and compared its promotion activity with that of 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). Female ICR mice, 7 weeks of age, were initiated with 100 μg of 7,12-dimethylbenz(*a*)anthracene, and were then topically promoted twice a week for 16 weeks with 100 mg of phytol or with 2.5 μg of TPA. In this model 95% of animals treated with phytol developed skin tumors within 16 weeks. The average number of lesions per mouse treated with phytol was significantly lower than that in mice treated with TPA, and this significant difference continued up to 16 weeks after the end of promotion treatment. Characterization of hyperplasia 48 h after topical application of agents showed that epidermal thickness and vertical thickness following topical application of phytol were significantly increased compared with vehicle controls, but were significantly smaller than in animals treated with TPA. Ornithine decarboxylase (ODC) activity following topical application of phytol was increased in a dose-dependent manner and showed a weak, delayed induction (which was maximal 11–12 h after treatment) as compared with the case of TPA. The specific binding of [^{3}H]phorbol-12,13-dibutyrate (PDBU) by JB6 cells was not inhibited by phytol at concentrations up to 1 m*M*. These results indicate that phytol has a weak tumor promoter activity compared to TPA and is a non-TPA-type tumor promoter in this model of mouse skin carcinogenesis.

Key words: Phytol — Non-TPA-type tumor promoter — Two-stage mouse skin carcinogenesis — Ornithine decarboxylase — Radioligand assay

Mouse skin tumorigenesis can be divided into distinct stages of tumor initiation and promotion. According to carcinogenesis experiments, tumor development on mouse skin can be provoked by topical application of an initiator, such as the polycyclic aromatic hydrocarbon 7,12-dimethylbenz(*a*)anthracene (DMBA), followed by repeated treatment with a tumor promoter.[1, 2] Our current understanding of responses to skin tumor promoters has been derived primarily from studies with 12-*O*-tetradecanoylphorbol-13-acetate (TPA), which affects basal cells but not differentiating cells in mouse epidermis.[3] While TPA is the strongest and most widely studied skin tumor promoter, other chemical compounds are known to possess tumor-promoting properties in mouse skin. There have been many reports which have described TPA-type promoters, such as Roussin red methyl ester,[4] and teleocidin,[5] and non-TPA-type promoters, such as okadaic acid,[6] thapsigargin,[7, 8] and mirex.[9]

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol), the fatty acid side chain of tocopherol, is a branched, long-chain aliphatic alcohol contained in large quantities in plants and is a component of chlorophyll. In recent extensive studies, various cellular and biological effects of phytol have been demonstrated, such as its action as an agonist in hypoxia,[10] peroxisome proliferation in skin sebaceous glands[11] and in liver,[12] anti-inflammatory effects,[13] antispasmodic activity,[14] inhibition of efflux of creatine kinase,[15] and reduction of the duration of pentobarbital-induced sleep.[16] Clinically, phytanic acid, which is a derivative of phytol, is associated with Refsum's disease and a phytol-free diet can prevent the progression of that disease.[17, 18] However, thus far, there have been no reports describing the tumor-promoting activity of phytol.

While studying the mechanism of hair growth and seeking agents which possess a hair growth-promoting effect, we noticed the epidermal proliferative and hair growth-promoting activity of phytol in the mouse. In the present study, therefore, we have examined the carcinogenicity of phytol using a two-stage carcinogenesis protocol and have assayed the activity of ornithine decarboxylase (ODC), a rate-limiting enzyme in the biosynthesis of polyamines related to cell growth, transformation and differentiation. In addition, we have studied whether or not phytol is a TPA-type promoter using a [^{3}H]phorbol-12,13-dibutyrate (PDBU) binding assay. We show for the first time the tumor-promoting activity of phytol on ICR mouse skin.

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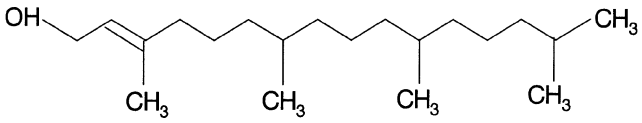

Fig. 1. Chemical structure of phytol. $C_{20}H_{39}OH$, MW 296.64.

MATERIALS AND METHODS

Subjects and chemicals Female ICR mice were obtained from Sankyo Labo Service Co. (Tokyo). Mice were housed in an air-conditioned room (22–23°C) with a light period of 6 AM to 6 PM. Food and water were available *ad libitum*. All mice were 7 weeks of age when the study started. RT101 cell line, which was derived from the BALB/c mouse epidermal JB6 cell, was obtained from American Type Culture Collection (Manassas, Virginia). DMBA, TPA, pyridoxal phosphate, dithiothreitol and L-ornithine hydrochloride were purchased from Wako Pure Chemical Inc. (Osaka). Phytol (shown in Fig. 1) was obtained from Tokyo Kasei Co. (Tokyo). Phorbol-12,13-dibutyrate was purchased from Sigma-Aldrich Japan (Tokyo). DL-[1-^{14}C]ornithine hydrochloride (50–62 mCi/mmol, 50 μCi/ml) and PDBU (10–20 Ci/mmol, 250 μCi/ml) were obtained from Amersham Life Science (Tokyo). All chemicals were of the highest purity commercially available.

Two-stage carcinogenesis protocol for mouse skin Panels of mice were subjected to a standard two-stage cutaneous chemical tumorigenesis protocol.[19, 20] The backs of the mice were clipped 3 days prior to the first treatment, and then shaved as necessary for the duration of the experiment. Mice were divided into five groups of 20 mice each for the initiation/promotion protocol (1: DMBA + phytol, 2: DMBA + TPA, 3: acetone + TPA, 4: acetone + phytol, 5: DMBA + ethanol). A single dose of 100 μg of DMBA was topically applied as an initiator in groups 1, 2 and 5; acetone was used as a vehicle control in groups 3 and 4. Phytol can not be dissolved in acetone, and we dissolved TPA and phytol in ethanol. From the results of preliminary experiments, topical application of phytol dissolved in ethanol induced tumors in a dose-dependent manner. A dose of 100 mg of phytol was subsequently used in these experiments and was applied to the shaved dorsal skin of mice in a volume of 0.2 ml using a micropipet 1 week after initiation in groups 1 and 4. TPA (2.5 μg) dissolved in ethanol was similarly used as a promoter in groups 2 and 3, while ethanol was used as a promoter control in group 5. Promotion treatments were given to all groups 2 days a week for 16 weeks. The appearance of animals was recorded by macrophotography once a week for 24 weeks after the end of promotion treatment. We counted the number of tumors on treated areas of the skin once every 4 or 8 weeks. Only tumors which had a diameter of 1 mm or greater, and which persisted for 2 weeks or longer, were recorded, as described previously.[21, 22] Thereafter, all mice were killed by cervical dislocation. Skin specimens were obtained from the backs of mice, fixed in 10% formalin, and then embedded in paraffin. Tissue slices 5 μm in thickness were prepared, and after hematoxylin-eosin staining, light microscopic observations were performed.

Hyperplasia study For the study of hyperplasia, backs of mice were carefully shaved at least 3 days prior to topical application of agents and treated with 100 mg phytol or 2.5 μg of TPA. Mice were killed 48 h later and their skins were removed. For each section of skin, the thickness of the epidermis from the basal layer to the stratum corneum was measured at ten equidistant interfollicular sites, and in addition, epidermal cell layers were also counted.[9, 23]

Determination of ODC At appropriate times after topical application of 100 mg of phytol or 2.5 μg of TPA to the dorsal skin of mice, the epidermis was separated from the dermis by brief heat treatment, followed by scraping with a razor blade.[24] Epidermal tissues were then homogenized and sonicated. Insoluble material was removed by centrifugation at 15,000*g* for 30 min and the supernatant fraction was used for estimation of ODC activity. The protein content of the supernatant was measured by using a Bio-Rad Protein Assay kit with bovine serum albumin as the standard, as described previously.[25] Enzyme activity was determined by measuring the release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine hydrochloride[24, 26] with minor modifications. Enzyme activity was expressed as nmoles CO_2/h/mg protein. Results of ODC activity are given as mean values for 3 or 4 mice.

Inhibition of [^{3}H]PDBU binding Inhibition of [^{3}H]-PDBU binding was assayed by the method described previously[27, 28] with minor modifications. RT101 cells, which are derived from mouse epidermal JB6 cell line and are sensitive to promotion of transformation by phorbol esters,[29, 30] were grown in 60 mm plastic culture dishes containing Dulbecco's modified Eagle's medium plus 10% fetal bovine serum. Subconfluent cultures (2 to 4×10^6 cells per dish) were washed 3 times with phosphate-buffered saline (PBS) and were preincubated in 2 ml of assay buffer containing bovine serum albumin at 1 mg/ml in PBS for 20 min at 37°C. Cultures were then washed again with cold PBS once prior to the addition of phytol (varied from 1 m*M* to 10 p*M*) and 10 n*M* [^{3}H]PDBU. Nonspecific binding was that observed in the presence of a large excess (30 μ*M*) of unlabelled PDBU. After this addition, cells were usually incubated for 1 h at 4°C and were then rapidly washed 3 times with ice-cold assay buffer. Cells were then solubilized for 30 min at 37°C in PBS containing 1% Triton X-100 and 0.25%

trypsin. Radioactivity was counted in 10 ml of ACSII scintillation fluid, and results reported are averages for three or four independent measurements.

Statistics Statistical analysis was carried out using Student's *t* test. *P* values of 0.05 or less were considered significant.

RESULTS

Two-stage carcinogenesis on mouse skin The results are summarized in Fig. 2. In the group treated with DMBA followed by TPA, skin tumors began to appear at 5 weeks, and 100% of animals had developed skin tumors within 13 weeks. In the group treated with DMBA followed by phytol, tumors started to appear at 7 weeks, and 95% of animals had developed skin tumors within 16 weeks. In the group treated with acetone followed by TPA, only 10% of animals developed skin tumors. In the two other groups of mice treated with acetone followed by phytol or treated with DMBA followed by ethanol, none showed any tumors arising during the experimental period.

The average number of lesions per mouse treated with DMBA followed by TPA was significantly increased compared with that in mice treated with DMBA followed by phytol immediately after the end of promotion treatment. This significant difference continued for the next 16 weeks of observation (Fig. 3). However, the average number of tumors in the group treated with DMBA followed by TPA, was decreased at 24 weeks after the end of treatment, whereas the average number in the group treated with phytol was essentially the same throughout the observation period. There was no difference in average number of tumors per mouse in either group 24 weeks after the end of the promotion treatment. None of the mice treated with DMBA+phytol or acetone+phytol died within 24 weeks during the experiment. So the dose of phytol does not seem to be a toxic one to mouse skin.

Macroscopic findings Well-demarcated papules or nodules, about 2 to 10 mm in diameter, with rough skin surfaces were diffusely observed on areas treated with DMBA followed by phytol and also on areas treated with DMBA+TPA. There were also several large-sized tumors with erosion or ulceration in their centers, which represent about 5% of all tumors on areas treated with DMBA+phytol.

Histological findings In the group treated with DMBA followed by phytol, acanthosis, hyperplasia and papilloma of the epidermis were seen. Rete ridges were elongated, and capillary dilatation and mononuclear cell infiltration were observed in the dermis (Fig. 4A). The peripheral layer of tumor cell nests showed a palisading arrangement. In several areas, tumor cells which had proliferated downward to the upper dermis showed some disorderly arrangement and nuclei often appeared atypical and pleomorphic. On the other hand, tumors, which had ulcerated

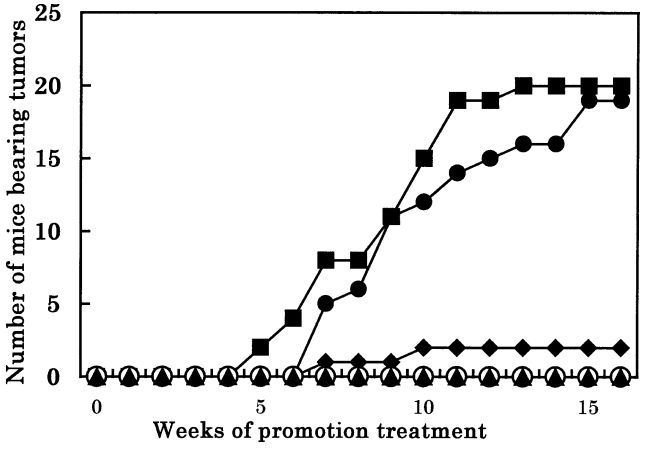

Fig. 2. Tumor-promoting activities of phytol and TPA. Groups were treated with DMBA and phytol (●), with DMBA and TPA (■), with acetone and TPA (◆), with acetone and phytol (○), or with DMBA and ethanol (▲). DMBA (100 μg) dissolved in 0.2 ml of acetone was topically applied once as an initiator and then 100 mg of phytol or 2.5 μg of TPA in 0.2 ml was topically applied as promoter 2 days a week for 16 weeks. Acetone and ethanol were used as controls of initiation and promotion treatment, respectively.

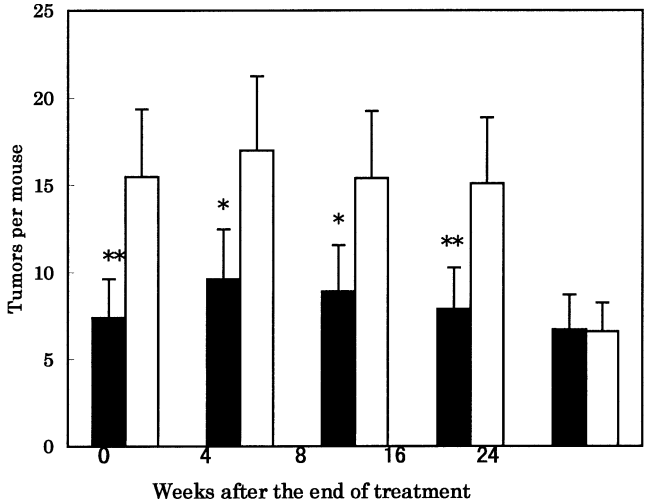

Fig. 3. Average number of tumors per mouse after the end of topical treatment. The average number of tumors per mouse treated with phytol was significantly decreased compared to those treated with TPA at 0, 4, 8, and 16 weeks after the end of treatment. However, there was no significant difference in the number of tumors in both groups at 24 weeks. * $P<0.05$, ** $P<0.01$. ■ DMBA+phytol, □ DMBA+TPA.

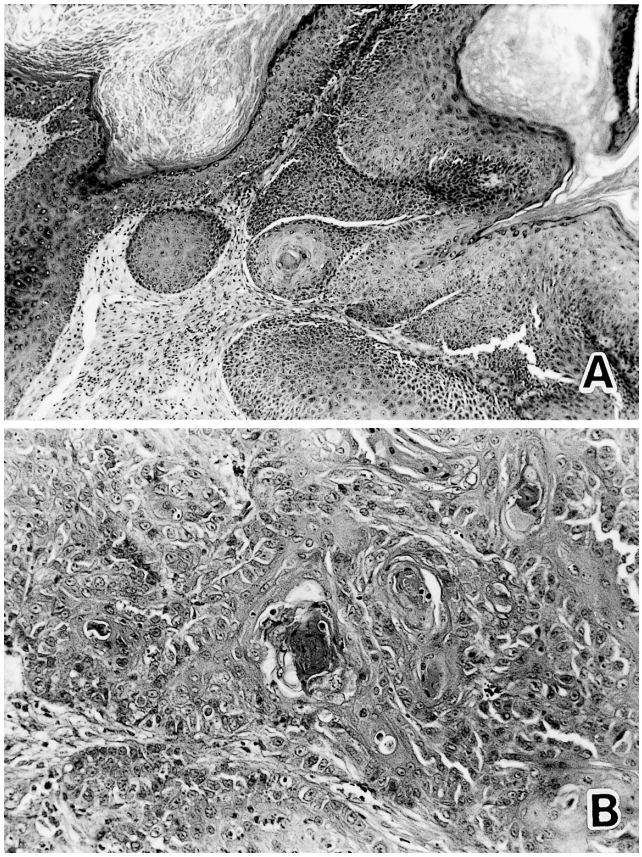

Fig. 4. Histological characteristics of tumors induced by topical application of phytol. A, Epidermis shows hyperkeratosis, acanthosis and papillomatosis. Tumor cells proliferated downward into the epidermis show a disorderly arrangement. Capillary dilatation and lymphocytic infiltration are observed in the dermis. HE, ×90. B, Tumors, which consist of irregular masses of epidermal cells, invade the dermis and subcutaneous tissue. Tumor cells are atypical and pleomorphic. The center of the tumor nest is occupied with eosinophilic materials. HE, ×180.

Table I. Results of Hyperplasia Study (Epidermal Thickness and Vertical Thickness According to Katiyar *et al*.[23])

Treatments[a)]	Epidermal thickness (μm)	Vertical thickness in terms of epidermal cell layers
Vehicle	20.8±0.7[b)]	1.8±0.2[b)]
Phytol	66.8±3.5[c,d)]	4.4±0.3[c,e)]
TPA	110.4±5.0[c)]	5.9±0.3[c)]

a) Animals were treated once with 0.2 ml of vehicle, 100 mg of phytol in 0.2 ml of vehicle or 2.5 μg of TPA in 0.2 ml of vehicle; 48 h after treatment mice were killed, and the skin was used for estimation of hyperplasia.
b) Mean±SE of 10 individual values; 2 skin biopsies were taken from each of 5 individual mice.
c) Significant vs. vehicle alone; $P<0.005$.
d) Significant vs. TPA; $P<0.005$.
e) Significant vs. TPA; $P<0.01$.

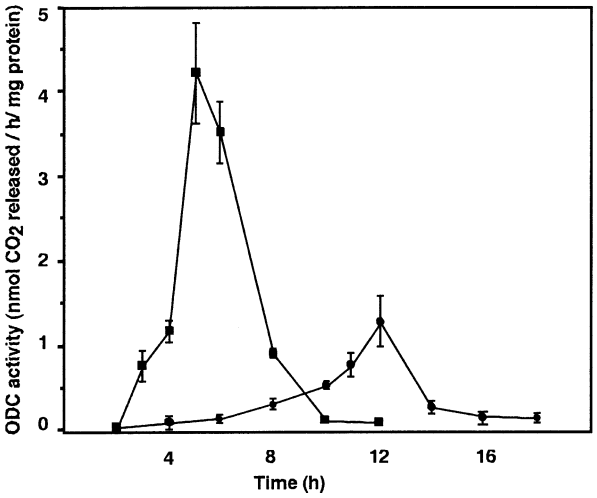

Fig. 5. Induction of ODC activity is shown in nmole CO_2 per mg protein per 1 h. The effect of a single topical application of 100 mg of phytol (●) or 11 μg of TPA (■) on the activities of epidermal ODC. Mice were treated with either TPA or phytol in 0.2 ml of acetone and killed at the time indicated. The activity of ODC was maximal about 11–12 h after the topical phytol treatment.

macroscopically, showed tumor cells consisting of irregular masses of epidermal cells invading the dermis and subcutis. Horn pearls were present in fairly large numbers in tumor nest centers, in which many atypical nuclei and mitotic figures were observed. The center of each nest was occupied with eosinophilic or keratotic materials and mononuclear cell infiltrates (Fig. 4B). Phytol and TPA showed about the same potency for production of skin papillomas and carcinomas.

Hyperplasia study As shown in Table I, within 48 h of TPA application, there was a significant increase in parameters of mean epidermal thickness and mean vertical thickness in terms of epidermal cell layers, when compared with the skin of vehicle-treated animals. Within 48 h following application of phytol, there were significant differences in parameters compared to vehicle- and TPA-treated skin. Acute histological examination of skin that had been treated with phytol showed inter- or intracellular edema of the epidermis and dermis that was milder in degree compared to skin treated with TPA.

ODC activity Fig. 5 shows the time course of ODC induction following topical application of phytol compared with TPA. TPA treatment rapidly increased ODC activity, which reached a peak about 5 h after application. In contrast to ODC induction by TPA, phytol caused no change for 5 h after treatment, then ODC activity slowly increased, reaching a peak after 11 h which was lower than that elicited by TPA; this level gradually returned to

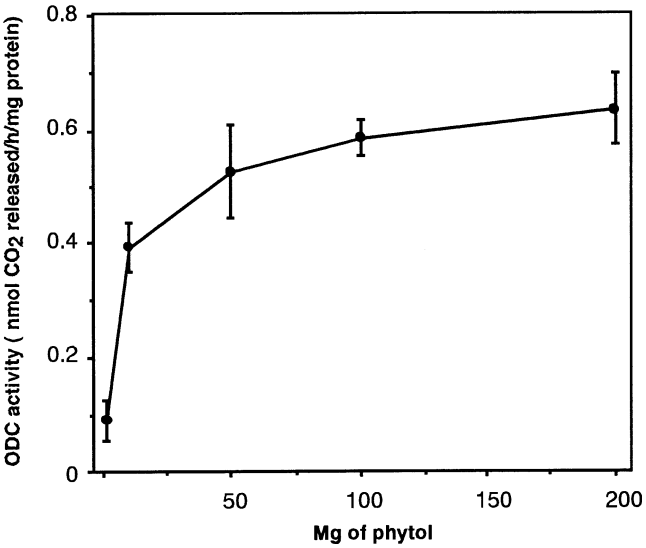

Fig. 6. Dose-response curve of ODC induction by phytol. ODC was assayed 11 h after applying various amounts of phytol up to 200 mg per mouse. The activity of ODC increased in a dose-dependent manner.

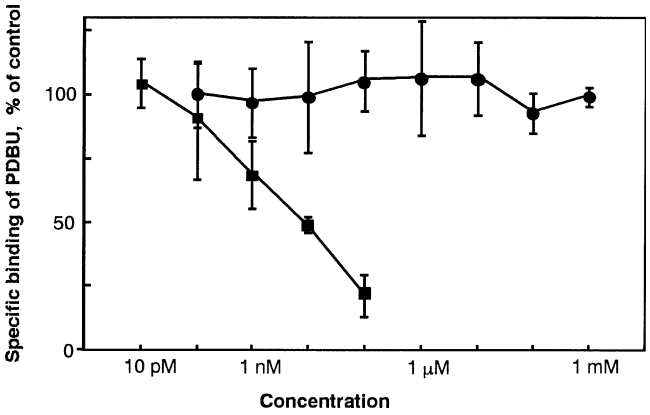

Fig. 7. Inhibition of [^{3}H]PDBU binding to cultured mouse epidermal JB6 cells. Specific binding of [^{3}H]PDBU to cells without the addition of phytol or TPA is taken as 100%. The specific binding of [^{3}H]PDBU is not inhibited by phytol at concentrations up to 1 m*M*, whereas TPA inhibits this binding in a dose-dependent manner. ● phytol, ■ TPA.

the control level by 18 h after treatment. The effect of various doses of phytol on the enzyme activity is shown in Fig. 6. The activity of ODC 11 h after topical application was increased in a dose-dependent manner at concentrations up to 200 mg per mouse.

Inhibition of [^{3}H]PDBU binding [^{3}H]PDBU binding to mouse epidermal JB6 cells was not inhibited by phytol at concentrations up to 1 m*M*. In contrast, TPA inhibited this binding in a dose-dependent manner at concentrations greater than 100 p*M* (Fig. 7).

DISCUSSION

Recently, various pharmacological effects of phytol have been reported. In particular, α-tocopherol, which is structurally related to phytol, has been shown to inhibit smooth muscle cell proliferation[31] and to suppress arachidonic acid metabolism.[32] Moreover, Tomita[33] showed that phytol might contribute to the prevention of cancer by augmenting immunological responses against tumor cells in early stages of carcinogenesis. Thus, vitamin E and related compounds have generally been regarded as inhibitors of cancer. In addition, Shimizu and Tomoo[13] have demonstrated an anti-inflammatory effect of phytol.

In the present study, we have for the first time demonstrated that phytol is a potent tumor promoter for DMBA-initiated mouse skin. There have been no previous reports describing the tumor-promoting activity of phytol and further, the chemical structure of phytol is not similar to that of any other tumor promoter previously described, although alkanes such as *n*-dodecane, which have some structural similarity to phytol, have tumor-promoting properties.[34] It is of interest that Elmets *et al.*[22] demonstrated a significant difference in rates of cutaneous tumorigenesis between different strains of mice and moreover, biphasic effects on cell proliferation and growth inhibition by tumor promoters, such as thapsigargin,[35] okadaic acid,[36] and TPA,[37] have been reported. Thus, it is likely that differences in dose, administration route or mouse strain influence tumorigenesis induced by phytol.

We found that 95% of animals treated with phytol developed skin tumors, while 100% of animals treated with TPA developed skin tumors. In addition, topical application of phytol alone did not provoke any tumors. Moreover, the average number of tumors per mouse treated with phytol was significantly smaller than that generated by TPA. Based on these data, we suggest that phytol is a weaker tumor promoter than TPA, and possesses only promoting, not initiating, activity. Interestingly, the average number of tumors generated by TPA treatment decreased as time passed, whereas the average number of tumors following phytol treatment was relatively stable throughout the experiment. These results indicate that tumor induction by TPA is reversible, whereas that by phytol is not. The cause of this difference is unknown, but it appears that the biological characteristics of tumors induced by phytol are different from those of tumors induced by TPA.

Many TPA- and non-TPA-type tumor promoters induce epidermal hyperplasia and acute inflammatory reactions,

though such changes are strongest following application of TPA itself.[6, 9, 38, 39] As shown in our hyperplasia study, phytol induces mild epidermal reactions (like other tumor promoters) compared with those of TPA, even though the concentration of phytol used topically was much higher than that of TPA. These findings indicate that phytol is a weaker promoter than TPA, as shown in the two-stage carcinogenesis study, and has acute inflammatory effects on both the epidermis and the dermis of mouse skin.

ODC is the first, and generally is regarded as the rate-limiting, enzyme in the biosynthesis of various polyamines.[40] Normal cell growth or proliferation seems to depend on the maintenance of critical levels of polyamines. Moreover, ODC and polyamines are induced in embryonic tissues, regenerating liver and neoplastic tumors.[41] As clearly shown in our study, phytol maximally induces ODC activity later (about 11–12 h) after treatment compared with TPA. In general, TPA-type promoters, such as TPA,[23] and teleocidin,[42] and non-TPA-type promoters, such as okadaic acid,[6] and calyculin A,[43] rapidly induce ODC activity within 4–6 h after a single topical application. In contrast, several non-TPA-type tumor promoters such as benzoyl peroxide,[44] anthralin,[45] and chrysarobin[46] show delayed induction with the peak of ODC activity at 24, 48 and 56 h after treatment, respectively. The cause of the delay in the induction of ODC by a single topical application of such tumor promoters is unclear. However, the peak of ODC activity shifts to earlier times and resembles that of TPA after multiple topical treatments.[45–47] Furthermore, tumor promoters, including phytol, might only affect basal cells but not differentiating cells, as does TPA.[3] Thus, delayed induction of ODC by phytol may be closely related to delayed exposure of basal cells, or alternatively, the signal transduction pathway that phytol stimulates might take longer to operate than that affected by TPA.

The results of the PDBU binding assay indicate that phytol is a non-TPA-type promoter. The mechanisms of action of TPA-type promoters are known to involve binding to phorbol ester receptors and down-regulation of protein kinase C.[48, 49] On the other hand, the mechanisms of action of several non-TPA-type tumor promoters are still unclear. It is of interest that some non-TPA-type promoters do not induce translocation of protein kinase C,[9, 50–52] while others activate protein kinase C.[53, 54] It seems that different pathways of signal transduction from membrane to cytosol are affected by different non-TPA-type promoters, and we are now further investigating the signal transduction pathway modulated by phytol.

In conclusion, we have demonstrated for the first time that phytol has a non-TPA-type tumor-promoting activity in addition to its various physiological effects, such as suppression of cell proliferation, induction of inflammation and spasmodic activity. Further studies on its mechanism of action as a non-TPA-type promoter should yield interesting results.

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REFERENCES

1) Yuspa, S. H., Hennings, H. and Saffiotti, U. Cutaneous chemical carcinogenesis: past, present, and future. *J. Invest. Dermatol.*, **67**, 199–208 (1976).
2) Yuspa, S. H. Cutaneous chemical carcinogenesis. *J. Am. Acad. Dermatol.*, **15**, 1031–1044 (1986).
3) Lichti, U., Patterson, E., Hennings, H. and Yuspa, S. H. The tumor promoter 12-O-tetradecanoylphorbol-13-acetate induces ornithine decarboxylase in proliferating basal cells but not in differentiating cells from mouse epidermis. *J. Cell. Physiol.*, **107**, 261–267 (1981).
4) Liu, J.-G. and Li, M.-H. Roussin red methyl ester, a tumor promoter isolated from pickled vegetables. *Carcinogenesis*, **10**, 617–620 (1989).
5) Fujiki, H., Suganuma, M., Matsukura, N., Sugimura, T. and Takayama, S. Teleocidin from *Streptomyces* is a potent promoter of mouse skin carcinogenesis. *Carcinogenesis*, **3**, 895–898 (1982).
6) Suganuma, M., Fujiki, H., Suguri, H., Yokozawa, S., Hirota, M., Nakayasu, M., Ojika, M., Wakamatsu, K., Yamada, K. and Sugimura, T. Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate type tumor promoter. *Proc. Natl. Acad. Sci. USA*, **85**, 1768–1771 (1988).
7) Marks, F., Hanke, B., Thastrup, O. and Furstenberger, G. Stimulatory effect of thapsigargin, a non-TPA-type tumor promoter, on arachidonic acid metabolism in the murine keratinocyte line HEL30 and on epidermal cell proliferation *in vivo* as compared to the effects of phorbol ester TPA. *Carcinogenesis*, **12**, 1491–1497 (1991).
8) Jackson, T. R., Patterson, S. I., Thastrup, O. and Hanley, M. R. A novel tumour promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells. *Biochem. J.*, **253**, 81–86 (1988).
9) Moser, G. J., Meyer, S. A. and Smart, R. C. The chlorinated pesticide mirex is a novel nonphorbol ester-type tumor promoter in mouse skin. *Cancer Res.*, **52**, 631–636 (1992).
10) Kelly, M. J. Alpha-tocopherol as agonist in hypoxia. *J.*

Pharm. Pharmacol., **38**, 66–68 (1986).
11) Van den Branden, C., Vamecq, J., Wybo, I. and Roels, F. Phytol and peroxisome proliferation. *Pediatr. Res.*, **20**, 411–415 (1986).
12) Watanabe, T. and Suga, T. Effects of phytol, a branched, long-chain aliphatic alcohol, on biochemical values and on hepatic peroxisomal enzymes of rats. *Chem. Pharm. Bull. (Tokyo)*, **31**, 2756–2761 (1983).
13) Shimizu, M. and Tomoo, T. Anti-inflammatory constituents of topically applied crude drugs. V. Constituents and anti-inflammatory effect of Aoki, *Aucuba japonica* Thunb. *Biol. Pharm. Bull.*, **17**, 665–667 (1994).
14) Pongrayoon, U., Baeckstrom, P., Jacobsson, U., Lindstrom, M. and Bohlin, L. Antispasmodic activity of β-damascenone and E-phytol isolated from *Ipomoea pes-caprae*. *Planta Med.*, **58**, 19–21 (1992).
15) Phoenix, J., Edwards, R. H. T. and Jackson, M. J. Inhibition of Ca^{2+}-induced cytosolic enzyme efflux from skeletal muscle by vitamin E and related compounds. *Biochem. J.*, **257**, 207–213 (1989).
16) Tsuchiya, T., Tanida, M., Uenoyama, S. and Nakayama, Y. Effects of olfactory stimulation with jasmin and its component chemicals on the duration of pentobarbital-induced sleep in mice. *Life Sci.*, **50**, 1097–1102 (1992).
17) Hansen, E., Bachen, N. I. and Flage, T. Refsum's disease. Eye manifestations in a patient treated with low phytol low phytanic acid diet. *Acta Ophthalmol.*, **57**, 899–913 (1979).
18) Friedman, K. J. and Shapiro, S. S. Changes in sterol and phospholipid fatty acid composition in Refsum's disease fibroblasts grown in the presence of phytol. *Clin. Physiol. Biochem.*, **3**, 249–256 (1985).
19) Kulkarni, J., Lalitha, V. S. and Bhide, S. Weak carcinogenic effect of masheri, a pyrolysed tobacco product, in mouse skin tumor models. *J. Cancer Res. Clin. Oncol.*, **115**, 166–169 (1989).
20) Fujiki, H., Suganuma, M., Suguri, H., Yoshizawa, S., Takagi, K. and Kobayashi, M. Sarcophytols A and B inhibit tumor promotion by teleocidin in two-stage carcinogenesis in mouse skin. *J. Cancer Res. Clin. Oncol.*, **115**, 25–28 (1989).
21) Elmets, C. A., Khan, W. A., Klemme, J. C. and Mukhtar, H. Impaired immunological surveillance by 7,12-dimethylbenz(a)anthracene augments its skin tumorigenicity in C3H mice. *Biochem. Biophys. Res. Commun.*, **151**, 148–152 (1988).
22) Elmets, C. A., Zaidi, S. I. A., Bickers, D. R. and Mukhtar, H. Immunogenetic influences on the initiation stage of the cutaneous carcinogenesis pathway. *Cancer Res.*, **52**, 6106–6109 (1992).
23) Katiyar, S. K., Agarwal, R. and Mukhtar, H. Inhibition of tumor promotion in SENCAR mouse skin by ethanol extract of *Zingiber officinale* Rhizome. *Cancer Res.*, **56**, 1023–1030 (1996).
24) O'Brien, T. G., Simsiman, R. C. and Boutwell, R. K. Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor-promoting agents. *Cancer Res.*, **35**, 1662–1670 (1975).
25) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254 (1976).
26) Fujiki, H., Mori, M., Nakayasu, M., Terada, M. and Sugimura, T. A possible naturally occurring tumor promoter, teleocidin B from streptomyces. *Biochem. Biophys. Res. Commun.*, **90**, 976–983 (1979).
27) Horowitz, A. D., Greenebaum, E. and Weinstein, I. B. Identification of receptors for phorbol ester tumor promoters in intact mammalian cells and of an inhibitor of receptor binding in biologic fluids. *Proc. Natl. Acad. Sci. USA*, **78**, 2315–2319 (1981).
28) Chida, K. and Kuroki, T. Presence of specific binding sites for phorbol ester tumor promoters in human epidermal and dermal cells in culture but lack of down regulation in epidermal cells. *Cancer Res.*, **43**, 3638–3642 (1983).
29) Colburn, N. H., Former, B. F., Nelson, K. A. and Yuspa, S. H. Tumor promoter induces anchorage independence irreversibly. *Nature*, **281**, 589–591 (1979).
30) Colburn, N. H., Wendel, E. J. and Abruzzo, G. Dissociation of mitogenesis and late-stage promotion of tumor cell phenotype by phorbol esters: mitogen-resistant variants are sensitive to promotion. *Proc. Natl. Acad. Sci. USA*, **78**, 6912–6916 (1981).
31) Boscoboinik, D., Szewczyk, A., Hensey, C. and Azzi, A. Inhibition of cell proliferation by α-tocopherol. Role of protein kinase C. *J. Biol. Chem.*, **266**, 6188–6194 (1991).
32) Pentland, A. P., Morrison, A. R., Jacobs, S. C., Hruza, L. L., Hebert, J. S. and Packer, L. Tocopherol analogs suppress arachidonic acid metabolism via phospholipase inhibition. *J. Biol. Chem.*, **267**, 11578–11584 (1992).
33) Tomita, Y. Immunological role of vitamin A and its related substances in prevention of cancer. *Nutr. Cancer*, **5**, 187–194 (1983).
34) Baxter, C. S. and Miller, M. L. Mechanism of mouse skin tumor promotion by n-dodecane. *Carcinogenesis*, **8**, 1787–1790 (1987).
35) Harmon, C. S., Ducote, J. and Xiong, Y. Thapsigargin induces rapid, transient growth inhibition and c-fos expression followed by sustained growth stimulation in mouse keratinocyte cultures. *J. Invest. Dermatol.*, **107**, 188–194 (1996).
36) Metaye, T., Sarrouihe, D., Kraimps, J. L., Barbier, J. and Begon, F. Inhibitions of protein kinase and protein phosphatases have opposite effects on thyrotropin-stimulated cAMP accumulation in human thyroid cells. *Cell. Mol. Biol.*, **42**, 547–556 (1996).
37) Yuspa, S. H., Ben, T., Patterson, E., Michael, D., Elgio, K. and Hennings, H. Stimulated DNA synthesis in mouse epidermal cell cultures treated with 12-O-tetradecanoyl-phorbol-13-acetate. *Cancer Res.*, **36**, 4062–4068 (1976).
38) Hakii, H., Fujiki, H., Suganuma, M., Nakayasu, T., Tahira, T., Sugimura, T., Scheuer, P. J. and Christensen, S. B. Thapsigargin, a histamine secretagogue, is a non-12-O-tet-

radecanoylphorbol-13-acetate (TPA) type tumor promoter in two-stage mouse skin carcinogenesis. *J. Cancer Res. Clin. Oncol.*, **111**, 177–181 (1986).
39) Fujiki, H., Suganuma, M., Nakayasu, M., Hakii, H., Horiuchi, T., Takayama, S. and Sugimura, T. Palytoxin is a non-12-O-tetradecanoylphorbol-13-acetate type tumor promoter in two-stage mouse skin carcinogenesis. *Carcinogenesis*, **7**, 707–710 (1986).
40) Pegg, A. E. and McCann, P. P. Polyamine metabolism and function: a review. *Am. J. Physiol.*, **243**, c212–c221 (1982).
41) Tabor, C. W. and Tabor, H. Polyamines. *Annu. Rev. Biochem.*, **53**, 749–790 (1984).
42) Fujiki, H., Mori, M., Nakayasu, M., Terada, M., Sugimura, T. and Moore, R. E. Indole alkaloids: dihydroteleocidin B, teleocidin, and lyngbyatoxin A as members of a new class of tumor promoters. *Proc. Natl. Acad. Sci. USA*, **78**, 3872–3876 (1981).
43) Suganuma, M., Fujiki, H., Furuya-Suguri, H., Yoshizawa, S., Yasumoto, S., Kato, T., Fusetani, N. and Sugimura, T. Calyculin A, an inhibitor of promotion phosphatases, a potent tumor promoter on CD-1 mouse skin. *Cancer Res.*, **50**, 3521–3525 (1990).
44) Binder, R. L., Volpenhein, M. E. and Motz, A. A. Characterization of the induction of ornithine decarboxylase activity by benzoyl peroxide in SENCAR mouse epidermis. *Carcinogenesis*, **10**, 2351–2357 (1989).
45) Dawson, M. I., Chao, W. R. and Helmes, C. T. Inhibition by retinoids of anthralin-induced mouse epidermal ornithine decarboxylase activity and anthralin-promoted skin tumor formation. *Cancer Res.*, **47**, 6210–6215 (1987).
46) Kruszewski, F. H. and DiGiovanni, J. Alterations in epidermal polyamine levels and DNA synthesis following topical treatment with chrysarobin in SENCAR mice. *Cancer Res.*, **48**, 6390–6395 (1988).
47) Kruszewski, F. H., Chenicek, K. J. and DiGiovanni, J. Effect of application frequency on epidermal ornithine decarboxylase induction by chrysarobin in SENCAR mice. *Cancer Lett.*, **32**, 263–269 (1986).
48) Fujiki, H., Tanaka, Y., Miyake, R., Kikkawa, U., Nishizuka, Y. and Sugimura, T. Activation of calcium-activated, phospholipid-dependent protein kinase (protein kinase C) by new classes of tumor promoters: teleocidin and debromoaplysiatoxin. *Biochem. Biophys. Res. Commun.*, **120**, 339–343 (1984).
49) Nishizuka, Y. The role of protein kinase C in cell surface signal transduction and tumor promotion. *Nature*, **308**, 693–698 (1984).
50) Perchellet, E. M., Gali, H. U., Gao, X. M. and Perchellet, J. P. Ability of non-phorbol ester-type tumor promoter thapsigargin to mimic the stimulatory effects of 12-O-tetradecanoylphorbol-13-acetate on ornithine decarboxylase activity, hydroperoxide production, and macromolecule synthesis in mouse epidermis *in vivo*. *Int. J. Cancer*, **55**, 1036–1043 (1993).
51) Kumar, R. and Holian, O. Inhibition of mouse skin protein kinase C by benzoyl peroxide. *J. Invest. Dermatol.*, **96**, 490–494 (1991).
52) Matsui, M. S., Mintz, E. and DeLeo, V. A. Effect of benzoyl peroxide on protein kinase C in cultured human epidermal keratinocytes. *Skin Pharmacol.*, **8**, 130–138 (1995).
53) Gopalakrishna, R., Chen, Z. H. and Gundimeda, U. Non-phorbol tumor promoters okadaic acid and calyculin-A induce membrane translocation of protein kinase C. *Biochem. Biophys. Res. Commun.*, **189**, 950–957 (1992).
54) Wang, X. J., Warren, B. S., Rupp, T., Beltran, L. M. and DiGiovanni, J. Loss of mouse epidermal protein kinase C isozyme activities following treatment with phorbol ester and non-phorbol ester tumor promoters. *Carcinogenesis*, **15**, 2795–2803 (1994).