ABSTRACT

The present study was designed to determine the effects of dietary 13-cis-retinoic acid and retinyl palmitate on mouse skin tumor promotion by 12-O-tetradecanoylphorbol-13-acetate (TPA). Female CD-1 mice were initiated with 150 nmol of 7,12-dimethylbenz(a)anthracene and promoted twice weekly with 8 nmol of TPA. Diets supplemented with retinyl palmitate to yield 60,000 or 200,000 IU or 700,000 IU for 5 wk followed by 350,000 IU per kg of diet resulted in no reduction in papilloma yield but did result in a dose-dependent decrease in the tumor burden (weight of tumors per mouse). Therefore, dietary retinyl palmitate yielded a dose-dependent inhibition of the number and weight of tumors promoted by TPA, whereas dietary 13-cis-retinoic acid resulted in a decrease in weight but not in number of tumors promoted by TPA.

RESULTS

Inhibition by Dietary Retinyl Palmitate of Skin Tumors Promoted by TPA. Dietary retinyl palmitate fed during promotion with TPA to DMBA-initiated mice inhibited the formation of skin papillomas in a dose-dependent fashion, as shown in Fig. 1. Retinyl palmitate at concentrations of 60, 200, or 350 IU/g of diet inhibited the number of papillomas/mouse by 13, 32, and 48%, respectively. Analysis of variance was performed on the results at 16 wk of promotion were analyzed to represent the preventive activity of dietary retinyl palmitate. At this time there was a significant difference between the groups treated with 350,000 IU of retinyl palmitate and the control group (P = 0.006). The difference between the group treated with 200,000 IU of RP and the control group verged on significance (P = 0.06). The values at 23 wk of promotion were considered to represent both preventive and therapeutic activity of dietary retinyl palmitate, because regression as well as prevention was evident at this time. The
numbers of papillomas/mouse in groups which received 200,000 or 350,000 IU of retinyl palmitate were significantly different from the value of the control group (P < 0.02). The rate at which mice became tumor bearers and the final number of papilloma-bearing mice were similar in control mice and in mice fed 60,000 to 200,000 IU of RP per kg of food, but was reduced (P < 0.05) in mice fed 350,000 IU of RP per kg of food (Fig. 1).

Influence of 13-cis-Retinoic Acid on Mouse Papilloma Promotion by TPA. Mice fed with a diet supplemented with 200,000 or 700,000 IU of CRA did not have a significantly different number of TPA-promoted tumors than did control mice, based on a 95% confidence level in the Student t test (Fig. 2). There was also no difference between CRA-treated and control mice in the rate at which mice became tumor bearers or in the final proportion of tumor bearers. However, CRA treatment resulted in a dose-dependent inhibition of the tumor burden per mouse, as measured by the weight of the tumors per mouse (Table 2). A dose-dependent inhibition of tumor burden was also found with dietary retinyl palmitate. The extent of inhibition was significant at all levels of retinoid supplementation beyond the basal diet component, based on the 95% confidence level of the Student t test.

Effect of Retinoids on Body Weight. Since caloric restriction can inhibit tumor promotion (12), mean food consumption and mean body weights during the promotion phase were determined, and the data are presented in Figs. 3 and 4. There was no statistically significant difference in food consumption between treated and control mice; however, there was a 16% difference in body weight between control mice and those fed at the highest concentration of RP. This influence may play a contributing role in the antitumor activity at the highest concentration of retinyl palmitate. However, this effect was titrated out at a dose level that still had a dramatic inhibitory effect on tumor promotion as assayed by tumor burden per mouse.

Toxicity of Retinyl Palmitate. Although mice fed with 700,000 IU of CRA per kg of diet appeared healthy throughout the study, mice fed with 700,000 IU of retinyl palmitate per kg of food displayed symptoms of toxicity: reduced body weight gain (insignificant by the Student t test); edema noted in the ears and around the eyes; bone deformities; and death. By the sixth wk of tumor promotion, 12 of 70 mice treated with the highest dose of RP had died from retinoid toxicity. At the fifth wk, the diet was adjusted so that this group of mice received ½ the dose it had been receiving, as is practiced in human clinics studying retinoid chemoprevention. With this diet adjustment, the deaths ceased. Thus, retinyl palmitate was significantly more toxic than was 13-cis-retinoic acid, at equal dose levels in the diet.

DISCUSSION

There was a dose response in CD-1 mice for the inhibition of TPA-induced mouse skin tumor promotion by dietary retinyl palmitate. Mice fed with a diet supplemented with retinyl palmitate to a level of 60,000 or 200,000 IU or 700,000 IU for 5 wk and then 350,000 IU per kg of diet demonstrated 9%,
with retinoic acid is not possible, although they gave roughly comparison of chemopreventive efficacy of retinyl palmitate/ig for the next 113 days, in the 3 RP groups, respectively. The basal diet contained 3,900 IU of retinyl palmitate per kg; this diet was supplemented in the test mice to yield the indicated amounts. Tumors from 15 mice of each group were removed and weighed at 21 wk of promotion. Figure 3. The effect of retinyl palmitate supplementation on body weight and food consumption. Mice were treated as described in Table 1. 37%, or 65% inhibition of papillomas/mouse, respectively, at 21 wk of promotion. Taking into account the biopotency of retinyl palmitate, 1.8 IU/μg (13), and the mean daily intake of food, the average daily consumption of retinyl palmitate was approximately 155 μg, 513 μg, or 1556 μg for 35 days and 1024 μg for the next 113 days, in the 3 RP groups, respectively.

Bollag (14) demonstrated that retinoic acid at a dose of 0.2 mg/g mouse given by stomach tube at intervals of 14 days during promotion by croton oil resulted in a 40% inhibition of papillomas at 20 wk of promotion. Using topical applications of 5.1 or 0.51 mg of retinoid acid per mouse twice weekly during promotion by TPA, Verma obtained a 78 or 61% inhibition of skin papillomas, respectively, at 19 wk of promotion (3). Due to the differences in experimental treatment, a quantitative comparison of chemopreventive efficacy of retinyl palmitate with retinoic acid is not possible, although they gave roughly similar results.

Caloric restriction has been shown to inhibit tumorigenesis (15). Boutwell (12) found that diet restriction inhibits mouse mammary tumorigenesis in Sprague-Dawley rats by 20%. In our experiment, it is possible that the inhibition of tumor promotion observed in the mice fed 350,000 IU of retinyl palmitate per kg of diet could be partly due to the 16% decrease in body weight. However, body weight was normal at the 200,000-IU/kg dose level of retinyl palmitate, and this dose level still yielded a marked reduction of tumor burden.

It is noteworthy that the slope of the line for papillomas/mouse became negative after 16 wk of promotion in the mice treated with the highest dose of RP. This decrease in papillomas at 16 wk was due to visible and palpable regression of tumors, not to deaths of animals bearing multiple papillomas. These results are consistent with a similar regression of papillomas observed by Bollag after 13 wk of retinoic acid treatment during croton oil-induced tumor promotion (14).

When 13-cis-retinoic acid was applied topically, it was almost as effective as retinoids in preventing the appearance of mouse skin papillomas promoted by TPA (3). However, dietary CRA did not prevent tumors from appearing, although it did reduce the weight of tumor burden per mouse. These results agree with those obtained by Verma (17) who also used CD-1 mice. Epstein reported preliminary studies in which 13-cis-retinoic acid p.o. did not reduce UV-induced skin cancer formation (18). There was no significant reduction in food consumption or body weight of mice fed diets supplemented with CRA, so that caloric restriction did not account for this reduction of tumor burden per mouse.

The levels and distribution of retinol and retinyl palmitate in selected tissues of the mice fed diet supplemented with RP were measured by high-performance liquid chromatography at 25 wk of promotion. The data have been published in a separate paper (19) and show that there was a dose-dependent increase in the accumulation of both RP and retinol in the tumor tissue and skin. Localized retinoids may induce differentiation within
papillomas, as they do with epidermal papilloma cells in vitro (20).

The possibility of increased prophylaxis during the late stages of carcinogenesis is of obvious clinical significance due to the growing recognition of preneoplastic lesions in many types of cancers. The quest for chemopreventive and therapeutic agents which are active at doses showing minimal toxicity warrants further investigation.

ACKNOWLEDGMENTS

We thank Lisa Werner, Lucia Tobin, and Yumiko Moriguchi for dietary preparation and animal care; Norma Seaver and Janet Gruhn for technical assistance; and Sally Anderson for expert secretarial assistance. We appreciate the assistance of Dr. E. M. Peng and Dr. D. Alberts in measuring the dietary retinyl palmitate and 13-cis-retinoic acid.

REFERENCES

Effects of Dietary Retinyl Palmitate or 13-cis-Retinoic Acid on the Promotion of Tumors in Mouse Skin

Helen L. Gensler, Ronald R. Watson, Satoru Moriguchi, et al.

Cancer Res 1987;47:967-970.

Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/47/4/967

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.