Inhibition of 7,12-Dimethylbenz(a)anthracene-induced Mammary Carcinogenesis by Retinyl Acetate

Richard C. Moon, Clinton J. Grubbs, and Michael B. Sporn

Summary

The administration of 2.5 mg retinyl acetate daily in the diet to female Sprague-Dawley rats beginning 7 days after the intragastric instillation of either 2.5, 5, or 15 mg 7,12-dimethylbenz(a)anthracene (DMBA) resulted in a reduction in the incidence of benign mammary tumors of 37, 30, and 31%, respectively. An equally significant reduction in the number of tumors was also evident. Although no difference was noted in the percentage incidence of mammary adenocarcinomas between the placebo and 2.5 mg retinyl acetate-treated groups at the 2.5-mg DMBA level, the percentage incidence was reduced by 52 and 39% in these groups at the 5- and 15-mg DMBA dose. Furthermore, the number of adenocarcinomas was also significantly reduced. Although both the percentage incidence and number of tumors were reduced by treatment with 1 mg retinyl acetate, these differences were not statistically significant. Liver histology and liver function tests of rats of the retinyl acetate groups did not differ from that of the control group. Similarly, the estrus cycle of treated animals did not differ from that of control rats. These data indicate that relatively large doses of retinyl acetate significantly inhibit the development of DMBA-induced mammary adenocarcinomas and benign tumors. Furthermore, the suppression of mammary tumorigenesis is apparently not the result of an alteration in either the metabolism of DMBA or estrogen nor to an inhibition of tumor growth resulting from retinyl acetate toxicity. The inhibitory effect of retinyl acetate may be related to the effect of retinoids on epithelial cell differentiation and/or reversal of carcinogen-induced anaplasia.

Introduction

It is well established that a principal function of vitamin A is to regulate the differentiation and maintain the integrity of several epithelial tissues (17, 24). Vitamin A or retinoid deficiency usually results in severe alterations of some epithelia (8, 24, 25). For example, the tracheal and bronchial epithelia of retinoid-deficient rats or hamsters develop keratinized squamous metaplasia with a loss of mucous secreting cells and the normal columnar ciliated epithelium (8). The development of such retinoid deficient squamous metaplasia is a process closely akin to that induced by certain chemical carcinogens (8). However, all epithelia do not respond similarly. The epidermis becomes hyperkeratotic, whereas the intestinal mucosa exhibits a decrease in the number of goblet cells, but exhibits no keratinization. Although these epithelia respond differently to the deficient state, the systemic administration of retinoids reverses the process and restores the epithelium to a normal functional capacity. Since retinoids have been shown to inhibit metaplasia of several epithelial tissues and since carcinogen-induced metaplasia appears to be similar to that resulting from retinoid deficiency, several investigators have attempted to reverse or prevent tumorigenesis by the administration of retinoids. Chu and Malmgren (4) have shown that the induction of squamous cell carcinomas by the topical administration of DMBA to the uterine cervix and vagina of hamsters can be inhibited if 10% retinyl palmitate is added to the vehicle containing the carcinogen. These same workers (4) also observed an inhibition of polycyclic hydrocarbon-induced squamous cell tumors of the esophagus and forestomach when retinyl acetate was added to the carcinogen. A protective effect of the retinoids has also been reported for chemically induced carcinoma of the hamster trachea and bronchi by Saffiotti et al. (21) and Port et al. (20), of the rat lung by Nettesheim et al. (18), and of the skin of mice by Bollag (1). On the other hand, a few workers have reported that retinoids are either ineffective or enhance experimentally induced carcinogenesis (22, 23). Only 2 reports relative to retinoids and mammary tumorigenesis have appeared in the literature. Schmahl et al. (22) reported that retinyl palmitate was ineffective (i.e., neither inhibitory nor stimulatory) in regard to DMBA-induced rat mammary tumorigenesis. However, Brandes and Anton (2) have shown that retinol enhances the effect of Cytoxan on the inhibition of mammary cancer in mice.

Since there is a paucity of data regarding retinoids and mammary tumorigenesis and since the data available are equivocal, a further investigation of this relationship appeared warranted.

Methods

Virgin female Sprague-Dawley rats (42 days old) were

1 Presented at the Conference "Early Lesions and the Development of Epithelial Cancer." October 21 to 23, 1975, Bethesda, Md. Supported in part by Contract NO1 CP23292 from the Division of Cancer Cause and Prevention, National Cancer Institute. The abbreviations used are: DMBA, 7,12-dimethylbenz(a)anthracene; BSP, Bromsulphalein; SGPT, serum glutamic pyruvic transaminase.

2 Presenter.
effect of retinyl acetate on incidence and number of mammary cancers induced by DMBA

<table>
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<th>Group</th>
<th>DMBA (mg)</th>
<th>Retinyl acetate (mg)</th>
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<th>Incidence (%)</th>
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* Active centers, total number of cancers in each group divided by total number of rats in group.

** Significant from respective DMBA controls at the 5% level or better.
the high dose retinyl acetate and either 5 or 15 mg DMBA and their respective placebo controls.

Benign tumors developed spontaneously in 2 of 50 rats receiving the retinyl acetate placebo and in 2 of 50 rats receiving 1 mg retinyl acetate daily (Table 2). However, no mammary tumors appeared spontaneously in the animals receiving 2.5 mg retinyl acetate daily. Again, as with the incidence of adenocarcinomas, the incidence of benign tumors was unchanged in the groups of rats receiving the low dose of retinyl acetate. On the other hand, animals receiving the high dose of the retinoid (2.5 mg) exhibited a percentage incidence that was significantly less than that of the placebo-treated animals. Furthermore, the number of active centers was reduced in all groups of rats receiving the high dose of retinoid as well as in those rats treated with either 2.5 or 5 mg DMBA and 1 mg retinyl acetate daily.

In Groups 1, 3, 9, and 12, in which liver function was evaluated, no significant differences in the levels of SGPT or serum bilirubin were apparent as a result of retinoid or a combination retinoid and carcinogen treatment. In addition, plasma BSP clearance did not differ between the groups, and liver histology was essentially the same in all groups. Furthermore, there was no appreciable difference in the average initial or terminal body weight between any of the retinoid treated groups and their respective controls. The percentage increase in body weight during the course of the experiment in Groups 1, 3, 9, and 12 was 74, 72, 78, and 85%, respectively. The greater gain in body weight in the retinoid treated groups and their respective controls.

Vaginal washing obtained from animals of Groups 1, 3, 9, and 12 over a period of 5 to 6 estrus cycles indicated that neither the retinoid nor carcinogen affects the estrus cycle since the animals of all groups exhibited normal 4 to 5-day cycles.

**Discussion**

Although the incidence of spontaneous mammary cancer in Sprague-Dawley rats is relatively low, a high incidence of mammary cancer may be induced in these animals by the i.g. administration of a single dose of DMBA. The incidence, latent period, growth rate, and number of mammary cancers induced with such hydrocarbons have been shown to vary with dosage, age, and endocrine status of the host (11). Optimal induction of mammary cancer in this strain of rats is achieved by an i.g. dosage of 20 mg DMBA administered at 50 to 65 days of age. A reduction in dosage results in a decrease in the incidence and number of mammary cancers (11). Under optimum conditions, the mean time of appearance of the first palpable tumor is approximately 42 days after administration of DMBA (5, 10). However, DMBA-induced mammary carcinoma has been detected histologically at 10 to 14 days after the administration of the carcinogen (5, 10). Although DMBA-induced mammary cancer is detectable as early as 10 days after feeding the carcinogen, initiation of the carcinogenic response is essentially complete within 24 hr following the administration of DMBA.

We have previously shown that maximum uptake of DMBA by mammary parenchymal cells occurs at approximately 6 hr after the i.g. administration of the compound (13). Furthermore, binding of DMBA to parenchymal cell protein and DNA is maximal at 16 hr postfeeding (14). Thus, in the present study, the initiation of retinoid treatment must have coincided with the presence of early DMBA-induced mammary lesions; i.e., after neoplastic transformation but prior to histologically discernible carcinoma.

The continuous daily ingestion of 2.5 mg of retinyl acetate definitely suppresses the development of mammary adenocarcinomas resulting from the administration of either 5 or 15 mg DMBA. On the other hand, the percentage incidence of mammary cancer in the groups of animals receiving 2.5 mg of DMBA and either retinyl acetate or the placebo were similar. This observation was probably due to the small number of animals (15 of 148) within these groups developing carcinoma within the time limits of the experiment. Furthermore, the number of cancers developing at this dose of carcinogen is relatively small (a total of 21), and the larger number of carcinomas in the high retinyl acetate groups was due solely to 1 animal possessing 7 cancers. In addition to the dramatic reduction in the percentage incidence in the number of tumors/no. of Active centers

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<th>Group</th>
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* Active centers, total number of tumors in each group divided by total number of rats in group.

* Significant from respective DMBA controls at the 5% level or better.

**Table 2**

Effect of retinyl acetate on incidence and number of benign tumors induced by DMBA.

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groups of animals receiving either 5 or 15 mg DMBA and retinyl acetate, an equally significant effect of the retinoid was evident in reducing the number of cancers in this group. Retinyl acetate not only inhibited DMBA-induced mammary carcinogenesis but also suppressed the development of benign tumors. As shown in Table 2, the administration of 2.5 mg of the retinoid not only resulted in a lower incidence but also affected a reduction in the number of benign tumors irrespective of the dosage of DMBA used. Since previous studies (14) have shown that initiation of tumorigenesis has already occurred prior to retinoid treatment, it must be assumed that retinyl acetate is in some manner either altering the further differentiation of the early lesion to carcinoma or inhibiting proliferation of the lesion to a macroscopic tumor.

Our data do not agree with those of Schmahl et al. (22), which showed that retinyl palmitate was ineffective in altering DMBA-induced tumorigenesis. Although the reason for the discrepancy between the 2 studies is unknown, one factor may be the mode of administration of the retinoids. In the present study, the retinoid was administered i.g. once each week. Ganguly and Krinsky (7) have shown that the retinyl ester level in the plasma peaks at 5 to 7 hr after the i.g. instillation of the retinoid and approaches that of the control level at approximately 24 hr after feeding the compound. Thus, it would appear that a sustained blood level of the retinoid was not attained in the experiments of Schmahl et al. (22), which could account for the discrepancy between the 2 studies. It is also of interest that Fretz et al. (6) had previously reported a mammary tumor incidence of 90% and an average induction time of 102 ± 64 days following 3 i.v. injections of 2 mg DMBA; however, in their retinoid study, control rats exhibited a 49% tumor incidence and an induction time of 230 ± 100 days at a DMBA dosage twice that used in their earlier study.

The mechanism by which retinyl acetate suppresses DMBA-induced mammary tumorigenesis is unknown. However, several factors may be ruled out. The inhibition of the metabolism of DMBA by liver hydroxylase or microsomal oxidases does not appear to be a factor, since uptake and binding of the carcinogen by the mammary parenchymal cell protein and DNA are complete prior to treatment with the retinoid (14). Such a mechanism could be a factor if retinoid treatment is begun prior to or concomitant with carcinogen treatment, since Hill and Shih (9) have found an approximately 40% inhibition of DMBA metabolism through inhibition of liver microsomal oxidases by retinyl acetate. Failure of tumor development and/or growth is not apparently due to a toxic effect of retinyl acetate, since body weight, liver histology, and the liver function tests (BSP clearance, SGPT, and serum bilirubin) did not differ between animals of the control (Group 1), retinyl acetate (Group 3), carcinogen (Group 9), or carcinogen and retinyl acetate (Group 12) groups. The possibility also exists that the inhibition of tumorigenesis in this study may have been due to an alteration in estrogen metabolism, since it is well documented that either a deficiency (5) or large amounts of estrogen (12) suppress the appearance of DMBA-induced tumors. Although estrogen levels were not determined, the observation that the estrus cycles of rats of the several groups (1, 3, 9, and 12) did not differ would seem to indicate that, if estrogen metabolism was affected, the change in metabolism was minor. Thus, estrogen metabolism in animals receiving the retinoid must have been within the physiological range.

Whether or not the retinoid is affecting epithelial differentiation or is affecting a reversal of carcinogen-induced proliferation and anaplasia, as has been shown by Chopra and Wilkoff (3) for the mouse prostate, is not readily apparent from this study. However, further studies along these lines, using retinyl acetate as well as other retinoids, should prove most informative.

Acknowledgments

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We also wish to express our gratitude to Dr. George M. McCormick, Louisiana State University Medical Center, Shreveport, La., for his assistance in evaluating the tumors histologically.

Recrystallized DMBA was provided by Dr. Marcia Litwack, Carcinogenesis Program, National Cancer Institute and Dr. Evelyn Murrill, Midwest Research Institute, Kansas City, Mo.

References


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