Inhibition of Rat Mammary Carcinogenesis by Short Dietary Exposure to Retinyl Acetate

David L. McCormick, Fredric J. Burns, and Roy E. Albert
Institute of Environmental Medicine, New York University Medical Center, New York, New York 10016

ABSTRACT

Previous work has indicated that prolonged exposure to retinyl acetate in the diet can inhibit mammary cancers in rats given the carcinogen 7,12-dimethylbenz(a)anthracene. This study was designed to determine whether retinyl acetate was an effective inhibitor when given for short periods at the time of and after the administration of the carcinogen. Virgin female Lewis rats were given 20 mg 7,12-dimethylbenz(a)anthracene in 1 ml sesame oil intragastrically at 50 days of age. The rats were fed Purina laboratory chow supplemented with 250 ppm retinyl acetate in groups of 20 according to the following schedule: —2 to +1 weeks; +1 to +30 weeks; +1 to +12 weeks; +12 to +30 weeks; —2 to +30 weeks; and none, where Time 0 was the day of 7,12-dimethylbenz(a)anthracene administration. Otherwise, animals were given standard Purina laboratory chow. At 30 weeks, groups receiving retinyl acetate from —2 to +1, +1 to +30, +12 to +30, and —2 to +30 all showed a significant decrease in tumor multiplicity in comparison to non-retinyl acetate-treated controls. The greatest decrease was seen in the longest treatment group (—2 to +30 weeks), but a nearly equal reduction was seen in the group receiving a short retinyl acetate exposure at the time of carcinogen availability (—2 to +1 weeks). In the +1 to +12 group, the inhibition of tumor development was temporary, inasmuch as tumor values returned to control levels by Week 30. These results indicate that retinyl acetate inhibition of mammary cancer is not limited to the late stage of the disease, because the retinoid was almost equally effective when given for a short period at the time of carcinogen availability.

INTRODUCTION

Chemical carcinogenesis in the rat mammary gland can be considered to consist of 2 phases: an early phase, involving the metabolic activation of the carcinogen and its interaction with the mammary parenchymal cells; and a second, or late, phase encompassing the growth and development of palpable tumors from cells that have been altered by the carcinogen.

Several studies have demonstrated that dietary retinyl acetate supplements can inhibit the second, or late, phase of DMBA-induced mammary carcinogenesis (5, 12). This inhibi-

tion has been achieved by beginning the retinoid supplement 7 days after a single dose of DMBA; at this time, the binding of the carcinogen to mammary parenchymal cell DNA and protein is complete, and unreacted DMBA is no longer detectable in the gland (8, 12). Comparatively little is known about possible inhibitory effects of retinoids on the early stage of mammary carcinogenesis and whether any effects on the early stage will alter the late stage inhibition.

Retinoids exhibit several biological actions that could influence mammary tumor growth and development. Vitamin A compounds have been shown to influence epithelial differentiation (14) and may act to arrest or reverse premalignant cells during their progression to a malignant lesion (12, 16). Influences on the immune system have been implicated, inasmuch as retinoids stimulate both cell-mediated (3, 10) and humoral (2) immune responses. Effects of retinoids on the mammary gland itself have been noted, because prolonged retinoid treatment inhibits mammary end-bud proliferation (13).

There is in vitro evidence suggesting that retinoids may also modify the first, or early, stage of carcinogenesis. Several retinoids inhibit the microsomal metabolism of benzo(a)pyrene, DMBA, and 3-methylcholanthrene to their proximal carcinogenic metabolites (7). Retinyl acetate treatment decreased induced aryl hydrocarbon hydroxylase activity in epithelial cell cultures (19), and retinoids have been shown to decrease carcinogen binding to DNA in epithelial cells (19) and human diploid fibroblasts (9). Vitamin A deficiency increased carcinogen binding to DNA in tracheal organ culture (4).

The purpose of the present experiment was to determine whether mammary tumors could be inhibited when retinoid exposure was limited to specific periods corresponding to early and late phases of carcinogenesis and to determine whether short-term retinoid exposure would be additive with the inhibition of carcinogenesis seen with long-term, postcarcinogen retinoid administration.

MATERIALS AND METHODS

Virgin female Lewis rats were obtained as weanlings from Microbiological Associates, Walkersville, Md., and housed 2 to a cage in a temperature (22 ± 1°)- and light-cycle (12 hr light, 12 hr dark)-controlled room. All animals were allowed free access to drinking water and Purina laboratory chow with or without a supplement of 250 ppm retinyl acetate (in gel beadlet form, courtesy Hoffmann-LaRoche Inc., Nutley, N. J.) as required by the protocol. Feeding the retinoid at this level in the diet provided an intake of approximately 3 mg retinyl acetate per animal per day.

Rats were assorted randomly into groups of 20 as follows, with the day of DMBA administration taken as Time 0: Group 1, control diet; Group 2, retinoid-supplemented diet for 2 weeks prior to and 1 week following carcinogen administration (—2 to
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achieved by retinyl acetate treatment beginning 1 week post-DMBA and continuing until the end of the experiment (+1 to +30 (Chart 2)). Limited retinyl acetate administration during the second phase [+1 to +12 (Chart 3)] induced a temporary suppression in number of palpable tumors (17), but tumors-per-rat values reached control levels by 30 weeks after DMBA administration.

The logrank analysis involves a comparison of curves throughout the experiment rather than data at any specific point in time. Although the tumors-per-rat values in the non-retinyl-acetate-treated and +1 to +12 retinyl acetate groups were very similar at 30 weeks, the curves departed sufficiently at earlier times for the logrank test to indicate a significant (p < 0.01) difference between them.

Retinyl acetate treatment was also effective when begun 12 weeks after DMBA [+12 to +30 (Chart 4)], a time when a few palpable tumors were already present in the group. The reduction in tumor yield in the +12 to +30 group was similar to, but slightly smaller than, that seen in the +1 to +30 group.

It was of interest to determine to what extent the combined effects of multiple short periods of retinoid administration (e.g., +1 to +12 and +12 to +30) were equal to the inhibition seen with the corresponding long period (e.g., +1 to +30). To make these comparisons, the tumors-per-rat values in the component (short) treatment groups were calculated as a fraction of control.  

RESULTS

Significant differences in tumor yield were seen in all groups receiving retinyl acetate supplements in comparison to dietary controls (p < 0.01). The greatest inhibitory effect of retinyl acetate occurred in the group receiving the longest treatment (−2 to +30); tumors-per-rat values were reduced to about 60% of control levels. An almost equivalent decrease, however, was achieved by the shortest treatment period (−2 to +1), a schedule which limited the retinoid supplement to approximately the time of carcogen availability (Chart 1).

Retinoid treatment in the second, or late, phase of carcinogenesis produced somewhat different results than did treatment in the early phase. Our data are similar to those of Moon et al. (5, 12), showing a significant reduction in tumor yield achieved by retinyl acetate treatment beginning 1 week post-DMBA and continuing until the end of the experiment [+1 to +30 (Chart 2)]. Limited retinyl acetate administration during the second phase [+1 to +12 (Chart 3)] induced a temporary suppression in number of palpable tumors (17), but tumors-per-rat values reached control levels by 30 weeks after DMBA administration.

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at each time point. Component values were then multiplied together to give an expected yield (as fraction of control) for treatment over the entire period. This expected fraction of control was then multiplied by number of tumors per rat seen in the control group at that time point, producing an expected tumors-per-rat value for the long treatment period.

A comparison of observed and expected tumor yields for the +1 to +30 groups is illustrated in Chart 2. It can be seen that the effects of retinoid treatment in the +1 to +12 and +12 to +30 groups do appear to be additive, inasmuch as the calculated and observed tumor values are almost identical throughout the experiment.

The same type of comparison is made for the –2 to +30 group in Chart 5. Chart 5 provides an expected tumor yield as calculated using the –2 to +1 (early-phase) and +1 to +30 (late-phase) retinoid treatment groups. The inhibitory effects seen in these short treatment groups do not appear to be additive; the observed –2 to +30 group shows a higher tumor yield than would be expected on the basis of the inhibition seen in the groups receiving retinoids for shorter periods.

It should be noted that variability in tumor response in this system does exist but is not greater than would be expected on the basis of random variation. Week 12 comparisons of control versus +12 to +30, and +1 to +30 versus +1 to +12 groups do show differences between groups, but the differences are within the S.E. of the measurements.

With the exception of a single fibroadenoma, all tumors were histologically classified as malignant. Over 95% of the malignant tumors were adenocarcinomas; remaining tumors were papillary carcinomas or showed a mixed adenopapillary pattern. No nonmammary tumors were observed.

No retinoid toxicity was observed, as indicated by weight gains being virtually identical in all groups throughout the experiment (Table 1). No signs of hypervitaminosis A (14) were seen in any retinyl acetate-treated rats, and no perturbations in estrous cycles were found in any animals receiving retinyl acetate supplements.

DISCUSSION

Where parallel groups were included, the results from this experiment with Lewis rats are consistent with those previously reported using the Sprague-Dawley model. Chronic postcarcinogen retinyl acetate treatment (+1 to +30) significantly inhibited tumor induction (5, 12), while administering the retinoid for a limited period in the late phase of carcinogenesis and then removing it (+1 to +12) produced a temporary suppression of tumor development (17).

The reversal of retinoid inhibition in the +1 to +12 group was somewhat delayed; although the retinoid supplement was removed from the diet at Week 12, tumors-per-rat values did not begin to increase towards control levels until Week 20. This delay may be a function of 2 factors: retinyl acetate accumulation in the liver (13), followed by its release after the retinoid supplement was removed from the diet; and the latency period required for a tumor to grow to palpable dimensions.

The inhibition of carcinogenesis by retinyl acetate administration for a short period at the time of carcinogen availability (–2 to +1) is in contrast to the temporary inhibition seen when the retinoid was supplied for an even longer but limited period after the carcinogen (+1 to +12). This comparison suggests that retinyl acetate may act via different mechanisms at different phases of carcinogenesis. Persistence of the retinoid due to liver storage should have no influence on tumor incidence in the –2 to +1 group, because any inhibition of the late phase due to retinoid persistence after Week 1 will be reversible.

The reversibility of retinyl acetate-induced inhibition in the late phase of tumor development, as seen in the +1 to +12

![Chart 4. Effect of delay in late-phase retinyl acetate treatment on mammary tumor response; □, control diet; ▲, retinyl acetate, Weeks +12 to +30. Bars, S.E.](chart4.png)

![Chart 5. Comparison of calculated and observed mammary tumor yield in rats given retinyl acetate between Weeks –2 and +30; □, control diet; ▲, observed yield for retinyl acetate treatment, Weeks –2 to +30; ○, calculated yield based on observed –2 to +1 and +1 to +30 yields. Bars, S.E.](chart5.png)

![Table 1](table1.png)
group, indicates that the antitumor activity of retinyl acetate should be related to the length of time for which it is available in the late phase. It would therefore be expected that the calculated cumulative effects of the +1 to +12 and +12 to +30 groups should be approximately the same as that observed in the +1 to +30 group. This is indeed the case, as is seen in Chart 2.

When the retinoid was supplied with the carcinogen (−2 to +1), the degree of inhibition of carcinogenesis was approximately the same as that when the supplement was administered from +1 to +30. These 2 effects do not appear to be additive, however, since Chart 5 shows the observed tumor yield in the −2 to +30 group to be higher than would be expected were the effects of the −2 to +1 and +1 to +30 periods additive. This nonadditivity of retinoid inhibition over the entire interval encompassing both the early and late phases of mammary carcinogenesis is presently unexplained.

The data here have concerned retinyl acetate influence on tumor appearance, without regard to the persistence or regression of a mammary tumor. Haslam (6) found that about one-third of the mammary tumors induced in Lewis rats by administration of 20 mg DMBA regressed spontaneously. In the present study, 12% of the tumors regressed in the control group, as compared to 29% in the retinoid-treated groups (p < 0.05 by x² analysis). If regressing tumors are excluded, analysis of the data from this experiment shows a greater retinyl acetate inhibition of carcinogenesis than was observed when all tumors were counted. The pattern of tumor inhibition by the various retinyl acetate treatment schedules was the same whether all tumors or only persisting tumors were counted.

It is apparent that retinyl acetate treatment can inhibit both early and late phases of rat mammary carcinogenesis. In vitro evidence of retinoid-induced alterations of carcinogen metabolism and DNA binding indicate that these differences could be the mechanism for the early-phase inhibition. However, the influence of retinoids on mammary tissue is complex and may involve other changes, such as alterations in the maturation rate of the gland. These effects may alter susceptibility of the gland to the carcinogen and thereby decrease tumor response. It is interesting in this respect that the tumor inhibition produced by late administration of the retinoid was not additive with the inhibition produced by early administration. Further study will be required to determine whether early and late inhibition have a different mechanistic basis.

REFERENCES

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