Synergistic Effect of Chronic Prolactin Suppression and Retinoid Treatment in the Prophylaxis of N-Methyl-N-nitrosourea-induced Mammary Tumorigenesis in Female Sprague-Dawley Rats

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ABSTRACT

Two hundred forty Sprague-Dawley rats were treated i.v. with 2.5 or 1.25 mg of N-methyl-N-nitrosourea (MNU) per 100 g body weight at 50 and 57 days of age. At 60 days of age, rats given either dose were divided into 4 groups (30 rats/group) and treated as follows: Group 1, controls; Group 2, 0.4 mg 2-bromo-a-ergocryptine (CB-154) per 100 g body weight injected s.c. once daily; Group 3, retinyl acetate (328 mg/kg diet) fed daily; and Group 4, CB-154 and retinyl acetate treatments combined. Rats that received the 2.5-mg dose of MNU were treated for 129 days; those that received the 1.25-mg dose of MNU were treated for 175 days. The rats that were treated with the high dose of MNU were maintained without any treatment for an additional 13 weeks, after which they were sacrificed. The rats that were treated with the low dose of the carcinogen were sacrificed immediately after treatment. All rats were palpated once weekly for palpable mammary tumors. The number of rats with mammary tumors and the total number of mammary tumors at cessation of treatments were, respectively, as follows. MNU (2.5 mg): Group 1, 22 of 30 (73%); 82; Group 2, 11 of 30 (37%); 17; Group 3, 11 of 30 (37%); 19; Group 4, 2 of 30 (7%); 2. MNU (1.25 mg): Group 1, 8 of 30 (27%); 14; Group 2, 4 of 30 (13%); 5; Group 3, 3 of 30 (10%); 4; Group 4, 0 of 30, (0%). Thus, chronic CB-154 treatment or retinyl acetate feeding markedly reduced the percentage of rats bearing mammary tumors and the total number of mammary tumors. The combined treatments were superior to either treatment alone, inasmuch as mammary tumorigenesis was nearly completely blocked in the rats of Group 4 that received the 2.5-mg dose of MNU and was totally blocked in the rats of Group 4 that received the 1.25-mg dose of MNU. Retinyl acetate feeding or CB-154-induced prolactin suppression appear to be equally effective treatments in the prophylaxis of MNU-induced mammary tumorigenesis in rats; the combined modality, however, appears to be far superior than either treatment alone.

INTRODUCTION

In recent years, increased attention has been turned toward the experimental use of retinoids for the chemoprevention of carcinogenesis. The vitamin A analogs have been very effective in suppressing the incidence of a number of experimental epithelial neoplasms (7), including mammary carcinomas induced by MNU3 and DMBA4 in rodents (2, 3, 8). The retinoids are most efficacious when used shortly after carcinogen treatment; thus, they appear to act primarily at the level of the incipient neoplasm, and they are not particularly effective when administered to animals in advanced stages of the disease.

Prolactin is an important hormone for the development of polycyclic hydrocarbon-induced mammary carcinomas in rats, inasmuch as drug-induced suppression of this hormone shortly after carcinogen treatment results in a significant reduction in tumor incidence (9, 12). The effect of this pituitary peptide on the genesis of MNU-induced rat mammary carcinomas, a recently developed model which reportedly is more closely akin to human breast cancer than is the polycyclic hydrocarbon rat model (1), has not been reported. Thus, the purpose of this study is to ascertain whether or not prolactin is important in the genesis of MNU-induced rat mammary carcinomas and if so, whether chronic prolactin suppression in conjunction with retinoid treatment will be more effective than either treatment alone. An interaction of retinoid treatment and endocrine therapy has not heretofore been studied, although an inverse relationship between steroid and retinoid receptors in ovariodependent and -independent tumors has been reported (4).

MATERIALS AND METHODS

Two hundred forty female Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) were divided into 8 groups, housed in a temperature-controlled (24 ± 1 °C) and light-controlled (14 hr/day) room, and given a diet of Wayne laboratory meal (Allied Mills, Inc., Chicago, Ill.) and water ad libitum. The vitamin A content of the Wayne laboratory meal is 15 IU of the vitamin per g of ration. All rats were treated i.v. with either 1.25 or 2.5 mg MNU per 100 g body weight at 50 and 57 days of age. MNU (Ash Stevens, Inc., Detroit, Mich.) was dissolved in 0.9% NaCl solution (10 mg/ml) acidified (pH 4 to 5) with acetic acid. Pituitary prolactin secretion was suppressed by daily s.c. injections of CB-154 (0.4 mg/100 g body weight) beginning at 60 days of age. CB-154 solution was prepared by dissolving the ergot alkaloid in a minimal amount of 100% ethanol and diluting with 0.9% NaCl solution so that the final concentration was 2.0 mg CB-154 per ml. Control rats and rats fed the retinoid diet alone were given daily injections of the diluent only. Retinoid treatment was begun at 60 days of age and was accomplished by blending retinyl acetate into the stock diet in the form of stable gelatinized beadlets at a concentration of 328 mg retinyl acetate per kg diet. Control rats and rats treated with CB-154 alone were fed gelatinized beadlets without retinyl acetate. The rats that received the 1.25-mg dose of MNU (Groups 5 to 8) were treated for 175 days and then were sacrificed. The rats that received...
the 2.5-mg dose of MNU (Groups 1 to 4) were treated for 129 days; treatment was then discontinued, and the rats were maintained on a standard rat chow (Wayne Lab-Blox) for a period of 13 weeks.

Beginning 1 month after carcinogen treatment, all rats were weighed and palpated once weekly for mammary tumors. Blood was obtained from each rat in Groups 5 to 8 by decapitation and analyzed by radioimmunoassay for prolactin (National Institute of Arthritis, Metabolism, and Digestive Diseases rat prolactin radioimmunoassay kit). Ovaries, uteri, adrenals, and pituitaries were removed and weighed from rats of Groups 5 to 8. Inguinal mammary glands were excised from rats of Groups 5 to 8 and prepared for wholemount evaluation. Mammary tumors were removed from rats of Groups 1 to 8, stained with hematoxylin and eosin, and examined histologically. Mean differences between blood prolactin levels, mean latency period of mammary tumor appearance, and organ weights were evaluated statistically by Student's t test. Mammary tumor incidence was analyzed by \( \chi^2 \) analysis. For an outline of the experimental design, see Table 1.

RESULTS

Rats Treated with 2.5 mg MNU (Groups 1 to 4). Chronic suppression of prolactin secretion or retinoid feeding reduced by nearly four-fifths the total number of palpable mammary tumors and reduced by one-half the number of rats bearing mammary tumors (Table 1). Combined prolactin suppression and retinoid feeding nearly completely blocked the development of mammary tumors; only 2 of 30 rats developed a total of 2 mammary tumors. The mean latency period of mammary tumor appearance (days after carcinogen treatment) was not significantly affected by CB-154 or retinoid treatments. Histologically, nearly all the palpable mammary tumors were adenocarcinomas or papillary carcinomas. The histological types of tumors which developed during treatments were similar to those which developed after treatment. Upon cessation of retinoid plus CB-154 treatment, mammary tumors developed at a rate comparable to tumor development in the control group (Chart 1). The same phenomenon occurred upon cessation of treatments in the groups treated with CB-154 (Chart 2) and retinoid (Chart 3).

Rats Treated with 1.25 mg MNU (Groups 5 to 8). Chronic prolactin suppression or retinoid treatment reduced by approxi-
MNU-induced Mammary Tumorigenesis

Chart 1. Effect of CB-154 treatment on MNU-induced mammary tumorigenesis in female Sprague-Dawley rats. p < 0.005 for the number of mammary tumors for controls versus CB-154-treated rats.

Chart 2. Effect of CB-154 treatment on MNU-induced mammary tumorigenesis in female Sprague-Dawley rats. p < 0.005 for the number of mammary tumors for controls versus CB-154-treated rats.

Chart 3. Effect of retinyl acetate feeding on MNU-induced mammary tumorigenesis in female Sprague-Dawley rats. p < 0.005 for the number of mammary tumors for controls versus retinyl acetate-fed rats.

It has been previously reported that CB-154-induced inhibition of prolactin secretion has a marked inhibitory effect on the development and growth of polycyclic hydrocarbon-induced mammary tumorigenesis in female rats (9, 12). It appears that this pituitary peptide is also important in the developmental phases of MNU-induced mammary tumorigenesis as well. The number of rats bearing mammary tumors was reduced by 50% and total number of tumors was reduced by two-thirds to four-fifths in rats treated with CB-154 shortly after carcinogen treatment. An important role for prolactin in the developmental phases of this experimental mammary tumor model has not heretofore been reported. It is important to note, however, that the antitumorigenic effect of CB-154 treatment is apparent only during drug treatment. Approximately 2 weeks prior to cessation of treatment and when the treatment was discontinued, mammary tumor incidence began to increase (Chart 2). This slight increase in tumor incidence during the last 2 weeks of treatment suggests a selection process for prolactin-insensitive mammary tumors. A previous report has shown that ovariectomy of rats either before or shortly after MNU treatment also sharply reduced the number of mammary tumors (1). It appears, therefore, that the MNU-induced rat mammary tumor model is a hormonally responsive neoplasm qualitatively similar to the polycyclic hydrocarbon rat models.

Retinyl acetate feeding has previously been reported to suppress the development of MNU-induced mammary cancers in rats (3). Our results confirm this study. In addition, we have shown that upon cessation of retinyl acetate feeding, mammary tumor incidence rate is comparable to that observed in controls (Chart 3). Thus, the antitumorigenic activities of retinyl acetate were apparent only when the animals were fed the retinoid. It appears therefore, that the MNU-induced rat mammary tumor model is a hormonally responsive neoplasm qualitatively similar to the polycyclic hydrocarbon rat models.

Although chronic CB-154 treatment or retinoid feeding alone was effective in markedly reducing mammary tumor incidence, the combined modality was superior to either treatment alone.
The combined treatment completely blocked mammary tumorigenesis in rats treated with the low dose of MNU (1.25 mg) and only 2 mammary tumors developed in 30 rats treated with the higher dose (2.5 mg) of the carcinogen. To our knowledge, no one has previously attempted to enhance the antitumorigenic efficacy of retinoid feeding by concurrent endocrine therapy. It is clear from the results of our study that a synergism between these 2 distinctly different types of therapeutic modalities does occur in the MNU-induced rat mammary tumor model. It is also apparent that the antitumorigenic effect of the combined modality is effective only during treatments; upon cessation of treatments, the rate of mammary tumor development is comparable to placebo-fed control rats (Chart 1).

When one compares retinyl acetate feeding with chronic CB-154-induced prolactin suppression on the general well-being of the animal, it is clear that the ergot treatment is considerably superior to retinoid treatment. CB-154 is an effective specific inhibitor of prolactin secretion; it does not significantly interfere with other endocrine processes at the dose levels used in this study (6, 10). The ergot-treated rats resembled the control rats in every respect; the only major disparity was observed in the mammary gland. That is, there was a conspicuous reduction or absence of neoplasia in the prolactin-suppressed animals. [Prolactin-suppressed rats have enlarged ovaries but normal estrous cycles (11) because of decreased prolactin luteolysis.] In contrast, the retinyl acetate-fed animals had a slight but significant reduction in body weight gains and a general insalubrious condition characterized by mildly roughened coat, scaly tail, and pale liver. The reduced body weight gains observed in the retinoid-treated rats (13%) are believed to be of insufficient magnitude to significantly impede mammary carcinogenesis. Since the onset of retinoid treatment occurred 3 days after carcinogen treatment, it is very unlikely that the retinoids influenced hepatic catabolism of the carcinogen. Nevertheless, the degree to which the retinoid-induced mild insalubrity influenced the carcinogenic process in this study cannot be resolved at this time. Increased success in developing synthetic retinoid analogs that are less noxious than retinyl acetate yet possess comparable antitumorigenic qualities is essential if the vitamin A analogs are to have any significant practical application. Once these analogs are developed, and noted progress is being made in this area (5), combined endocrine and retinoid therapies in the prophylaxis of breast carcinogenesis in humans may be a reality.

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REFERENCES

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