Criteria for Evaluating Hormones in the 7,12-Dimethylbenz[a]-anthracene-induced Mammary Tumor-rat Experimental Chemotherapy System

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SUMMARY

Two criteria were used to measure the effectiveness of hormones in the therapy of mammary tumors induced in Sprague-Dawley rats by 7,12-dimethylbenz[a]anthracene. These were complete regression of all tumors in the host and duration of complete remission. Well-established, growing mammary tumors were treated with testosterone propionate or 2α-methyl-dihydrotestosterone propionate. There was no significant difference between the effects of the two androgens, at equivalent doses, with respect to production of complete remissions. However, the median for duration of complete remission in rats treated with 2α-methyl-dihydrotestosterone propionate was significantly greater than for testosterone propionate. The results suggest that duration of remission is a useful adjunct for disclosing differences between compounds with similar abilities to cause tumor regression.

INTRODUCTION

Huggins et al. (5, 7) described the rapid induction of hormone-sensitive mammary tumors in rats with a single dose of 7,12-dimethylbenz[a]anthracene (DMBA). These reports stimulated further investigation regarding the use of such tumors for the detection of compounds potentially useful in the treatment of human breast cancer. Descriptions of induction, growth characteristics, hormone-responsiveness and morphology of the mammary tumors have appeared (1, 2, 4, 8, 9, 14, 15, 17, 20, 21). The suitability of the DMBA-induced mammary tumors in the rat as an experimental chemotherapy system has also been reported (3, 4, 6, 10, 11, 17-19). Earlier reports from our laboratory described a stringent test system aimed to simulate closely the clinical situation. Therapy was instituted on well-established, growing tumors, and the experimental chemotherapy system stressed 2 criteria for evaluating the response to therapy: complete regression of all tumors in the host, and duration of complete remission. The present report extends previous observations and suggests that a test system utilizing such criteria might differentiate between 2 compounds with apparently similar antitumor effects, testosterone propionate (TP) and 2α-methyl-dihydrotestosterone propionate (2α-MDTP). A preliminary account has been presented (16).

MATERIALS AND METHODS

Induction and therapy of mammary tumors were carried out in Sprague-Dawley rats as described previously (17, 18). Steroids were generously supplied by the Endocrine Evaluation Branch, Cancer Chemotherapy National Service Center (CCNSC), National Cancer Institute, NIH, USPHS, Bethesda, Maryland. The dose was dissolved in 0.2 ml sesame oil and administered i.m. (alternate thigh muscles) once daily, 5 times per week for 8 weeks, unless otherwise specified, to rats with well-established DMBA-induced mammary tumors. Tumors were measured biweekly and recorded as averages of 2 perpendicular diameters. Host weight changes were noted at the same time. Tumor-bearing rats treated with sesame oil alone were observed routinely as controls for tumor growth. Completion of therapy was followed by an additional 4 weeks of observation, at the end of which results were evaluated.

Criteria for response to therapy, described previously in detail (18), involved the whole animal rather than the tumor masses separately. A rat was designated in complete remission only if examination failed to reveal any palpable tumors at the end of the 4th week posttreatment. Duration of remission commenced from the time all tumors in the host became impalpable. The remission was considered terminated when a new tumor appeared, or recurrence at an old site was noted. Death of the host spontaneously, or induced because of ulceration (ear tumors) or other causes, also terminated the remission. Student's “t” test was used in calculating statistical significance of difference.

RESULTS

Antitumor Effects. The relative effectiveness of the 2 androgens were compared with respect to the production of remission of the DMBA-induced tumors (Table 1). Rats bearing well-established, growing mammary tumors of about 1 cm in average diameter were treated with 60 mg TP or 2α-MDTP.
Effects of various dosages of TP and 2α-MDTP (NSC 12198) on DMBA-induced mammary tumors in rats. TP, testosterone propionate; 2α-MDTP, 2α-methyl dihydrotestosterone propionate; DMBA, 7,12-dimethyl benz[a]anthracene.

a Average weight gains at end of 12th week of experiment for all groups.

per week for 4 weeks or 8 weeks. Effects were evaluated at the end of the additional 4-week observation period. TP produced complete remission in 60% and 47% of the rats treated for 4 and 8 weeks, respectively. There was no significant difference between these percentages. The proportions of complete remission produced by the 4- and 8-week treatments with 2α-MDTP were 90% and 72%, respectively. These, too, were not significantly different from each other. In fact, there was no significant difference between % complete remission for TP and for 2α-MDTP at equivalent treatment schedules (Table 1).

All rats treated with androgens gained weight progressively. At equivalent doses, the % host weight changes for rats treated with 2α-MDTP were about twice those for TP-treated rats.

Further tests were made with 2α-MDTP over a range of doses to determine (a) the effect of dosage on production and duration of complete remission, and (b) the subsequent effect of such titrations on the use of complete remission as a parameter of therapeutic effectiveness. The doses which ranged from 3.75 to 30 mg/week were administered for an 8-week period. Results were evaluated at the end of the usual 4-week observation period (Table 2). For ease of comparison, data for the 60 mg/week dose (Table 1) are included. Complete remission produced by the various doses ranged from 72% (60 mg/week) to 41% (3.75 mg/week). Although the differences in % complete remission were not statistically significant, the relationship between dose of 2α-MDTP and proportion of rats showing complete remission is apparent in Chart 1. Host weight gains for hormone-treated animals were approximately the same at all doses, and were about twice as great as for the control rats (Table 2).

Duration of Complete Remission. All rats with complete remission (Tables 1, 2) were observed for the duration of tumor-free status, except for 11 of the 18 treated with 2α-MDTP at 60 mg/week for 8 weeks. These were used for other experiments involving surgical castration. Duration of remission, in weeks, was calculated from the time of complete regression of all tumors in the rat (1–4 tumors) to the appearance of a new tumor mass or to death of host. Complete remissions were effected between 2 and 12 weeks after initiation of treatment. The duration of complete remission was not related to the rapidity with which this condition was attained. Chart 2 shows duration of complete remission for each animal in the variously treated groups, and the medians for each group. The medians for duration of complete remission for the 2α-MDTP dose titration (3.75–60 mg/week for 8 weeks)
Chart 2. Duration of tumor-free status of individual rats treated i.m. with testosterone propionate or 2α-methyldihydrotestosterone propionate. Numbers in parentheses represent medians in weeks. TP, testosterone propionate; 2α-MDTP, 2α-methyldihydrotestosterone propionate.

Chart 3. Relation of dose of 2α-methyldihydrotestosterone propionate to median duration of remission.

were not significantly different from each other, there appeared to be a direct relationship between dose and median of duration of tumor-free status (Chart 3), at least in the range 15–60 mg/week.

**DISCUSSION**

The experimental chemotherapy system described previously (17, 18) and used here simulates the clinical condition in that it entails therapy of well-established primary tumors resembling the human variety in morphology and in hormone responsiveness. Others, including Segaloff (13), have used transplantable DMBA-induced mammary tumors, which also show response to hormonal manipulation. Despite the usefulness of the various systems and their inherent advantages, the question still remains whether the definitive animal tumor system which correlates with human mammary carcinoma has been established. Many experimental chemotherapy systems test degree of inhibition of tumor growth. This has not been a particularly revealing measurement in mammary adenocarcinomas. Therefore, in an attempt to obtain more pertinent information, different criteria were selected to evaluate the effectiveness of an experimental drug. Complete regression of all tumors and duration of remission were established as criteria because they represent types of clinical evaluation in which tumor regression and extension of lifespan are the desired goals.

In the present experiments, 2α-MDTP therapy was not significantly better than TP on the basis of % complete remission produced at equivalent doses (Table 1). However, when medians of duration of complete remission were compared, again at equivalent doses, those for 2α-MDTP were significantly greater. The dose titration for 8-week therapy with 2α-MDTP ranged from 3.75 mg/week to 60 mg/week, in 2-fold dilutions. Neither % complete remission nor median duration of complete remission produced by the doses varied significantly from each other. Apparently, tests at even lower doses or with larger animal populations would be necessary to show real differences. Nevertheless, there did appear to be direct relationships between % complete remission and dose (Chart 1), and between median duration of complete remission and dose (Chart 3).

Drug treatment differed from the usual clinical procedure for breast cancer in that it was not continued until relapse occurred but was stopped after the finite times mentioned. This arrangement provided the required data. However, continuous, non-finite therapy might provide additional salient information.

The data clearly indicate that a distinction between the antitumor potentials of TP and 2α-MDTP can be made on the basis of duration of tumor-free status. Hopefully, additional studies will substantiate the fact that in the induced mammary tumor-rat host system the criteria of complete regression and duration of remission can elicit meaningful differences between 2 closely allied and, apparently, equal agents. The 2 compounds have been tested extensively in the therapy of human breast cancer. From a review of the results reported by the Cooperative Breast Cancer Group (12), we gained the impression that 2α-MDTP had a somewhat higher objective remission rate in 2 of the 3 categories. However, it has been stated that there is no difference between the number of remissions produced by the 2 androgens (13). It is evident that further investigation is necessary to determine the correlation between the 2 systems, clinical and laboratory.

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