Presence of C-19 Steroids in Mammary Shionogi Carcinoma (SC 115) in Castrated Mice

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ABSTRACT

Intact and castrated male DD/S mice were inoculated with androgen-dependent cells (SC 115). All intact animals developed tumors after Day 12 of inoculation; however, six of seven castrated animals presented tumors 48 days postinoculation. The levels of steroids in both tumors were then examined. In castrated mice, dehydroepiandrosterone and androst-5-ene-3ß,17ß-diol levels were diminished by 30% and 70%, respectively, while the amounts of testosterone and androstenedione were reduced by more than 90%. Our data also demonstrate that androstane-3a,17ß-diol and androstane-3ß,17ß-diol were decreased to 60% and dihydrotestosterone decreased to 6% of their normal value, respectively. This latter level (0.48 nm) was sufficient to still effect a potent androgenic response in the tumor. Besides, a highly significant correlation was found in these tumors between various C-19 steroids (dehydroepiandrosterone and androstane-3a,17ß-diol, r = 0.97, P < 0.01), suggesting a possible conversion of C-19 precursors into potent androgens in the tumors. Determination of the plasma steroid levels in the castrated animals clearly confirmed that potent androgenic steroids and precursors were still in the circulation 3 days after castration. It thus appears that C-19 steroids from adrenal origin may be also involved in "independent" tumor growth.

INTRODUCTION

Extensive studies have been performed with the androgen-dependent Shionogi SC 115 mammary carcinoma developed by Minesita and Yamaguchi (1, 2). It has been shown that this tumor which rapidly grows in intact males contains androgen receptors (3). Proulx et al. (4) have recently reported that castration of male mice 3 days before inoculation with androgen-dependent tumor cells does not prevent but only delays the appearance of tumors. Since the SC 1152 tumor that regrew after androgen removal had been previously bilaterally gonadectomized 2 days before the inoculation. The growth of the tumors in each animal was estimated by measuring every 3 days their average area (product of the 2 longest perpendicular dimensions). Six surviving intact mice were sacrificed 3 wk after the inoculation, and the tumors were carefully excised of adhering fat and dead tissues. They were then weighed and kept frozen at −20°C. Forty-eight days postinoculation, 6 castrated animals presented tumors. They were sacrificed, and the same procedure for the isolation of tumors was followed. In the second experiment, 50 animals were equally separated in 2 groups, and 25 mice were bilaterally gonadectomized. Three days later, the animals were bled from the retroorbital plexus using capillary tubes, and blood samples were pooled 3 by 3 to obtain 6–8 samples of 1 ml of plasma in each.

Steroid Assays. Progesterone, DHEA, Δ4-diol, Δ4-dione, testoster-
one, DHT, 3α-diol, and 3β-diol were measured in tumor and plasma as described previously (10, 11). These steroids have not been further identified and should then be called "steroid-like material."

RESULTS

Normal mice presented with tumors by Day 12 in 100% of the animals inoculated with 106 viable Shionogi SC 115 tumor cells. In castrated mice, 6 of 7 animals presented tumors 48 days postinoculation, the first tumor having appeared at Day 32. One intact mouse died before sacrifice, and its tumor content could not be analyzed. Chart 1 illustrates the growth rate of SC 115 tumors in intact animals and in animals castrated 2 days before inoculation. It can be seen that a delay of 20 days occurs in the appearance of tumors in castrated animals. However, from Day 32, it seems that the growth rate of SC 115 tumors is not significantly different in the intact and castrated animals.
The levels of progesterone, testosterone, DHT, DHEA, Δ5-diol, Δ4-dione, 3α-diol, and 3β-diol in the tumors are shown in Chart 2. While the progesterone levels are comparable in intact and castrated mice, it can be seen that all C-19 steroids are decreased in tumors from castrated animals. While DHEA and Δ5-diol concentrations are diminished by 30% and 70%, respectively, the amounts of testosterone and Δ4-dione in the tumors grown in castrated animals are more than 90% (P < 0.01) reduced. Furthermore, castration markedly decreases the content of dihydrotestosterone (from 2.21 ± 0.25 to 0.14 ± 0.04 ng/g; P < 0.01), while the levels of 3α-diol and 3β-diol are only decreased by 40%. The relationship between the tumor steroid concentrations in castrated animals of DHEA and 3α-diol is depicted graphically in Chart 3. There is a highly significant positive correlation between these 2 C-19 steroids (r = 0.97, P < 0.001). Statistical analysis of other steroid levels in the tumor shows that there are also significant correlations for Δ5-diol and DHEA (r = 0.81, P < 0.02), Δ5-diol and testosterone (r = 0.94, P < 0.002), testosterone and 3α-diol (r = 0.85, P < 0.02), and finally 3α-diol and Δ4-diol (r = 0.80, P < 0.02).

When examining the plasma steroid levels in male DD/S mice, we have observed that castration causes a decrease in the plasma levels of all steroids except DHEA (Chart 4). However, while testosterone and Δ4-dione are reduced to less than 50 pg/ml, the concentrations of DHT, 3α-diol, and 3β-diol are only decreased by 50–60%.

**DISCUSSION**

The present findings clearly demonstrate that several C-19 steroids are still present in the plasma from castrated male DD/S mice. In fact, the concentration of dehydroepiandrosterone remains unchanged after castration, while those of androst-5-ene-3β,17β-diol as well as dihydrotestosterone and androstane-
3α,17β-diol are only reduced by 50%. It is most likely that the adrenal glands are the source of the residual steroids. This is in agreement with the finding that mouse adrenals can transform pregnenolone into C-19 steroids (14).

We have recently observed that androgen-dependent as well as androgen-independent Shionogi tumors are able to convert dehydroepiandrosterone and androst-5-ene-3β,17β-diol into potent androgens (9). In this previous study, we have in fact clearly demonstrated that Shionogi tumors contain 3β-hydroxysteroid Δ5-Δ4-isomerase which causes the conversion of C-19 steroids into potent androgenic steroids. Furthermore, in independent tumors, we and others (2) have reported that the 5α-reductase activity is markedly increased. Hence, in agreement with our study on the C-19 steroid metabolism (9), the present data thus strongly suggest that the Shionogi tumors in the presence of plasma dehydroepiandrosterone are able to accumulate potent androgens. Moreover, it should be mentioned that a concentration of dihydrotestosterone reduced to 6% of the normal value (from 7.6 to 0.48 ng) can still exert a potent androgenic action. The correlation observed between the tumor concentrations of androgens following removal of the testicular influence.

The marked difference in the survival recently reported by Bélanger et al. (17) in patients receiving the combined treatment with a pure antiandrogen and an agonist of LHRRH can at least partly be explained in such an important role of androgens of adrenal origin. In fact, the antiandrogens could block the action of potent androgens formed in the prostate. Such a transformation of dehydroepiandrosterone and androst-5-ene-3β,17β-diol has been already reported in this human tissue (18). In the present study, although the concentration of C-19 steroids from adrenal origin in mouse plasma is much less important than in the human, we could easily demonstrate the presence of potent androgens in the tumor tissue.

Our data indicate that C-19 steroids may be involved in "independent" tumor growth in agreement with the previous suggestion of Desmond et al. (8). In fact, it thus appears that some tumors called independent remain sensitive to androgens and grow more slowly due to the decreased endogenous level of androgens following removal of the testicular influence.

REFERENCES

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