Hormonally Responsive versus Unresponsive Progression of Prostatic Cancer to Antiandrogen Therapy as Studied with the Dunning R-3327-AT and -G Rat Adenocarcinomas

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ABSTRACT

The present study has compared the response to antiandrogen therapy of the serially transplantable Dunning R-3327-AT (hereafter called AT) versus Dunning R-3327-G (hereafter called G) rat prostatic adenocarcinoma. Castration or chemical antiandrogen therapy (i.e., cyproterone acetate and diethylstilbestrol) of rats bearing established AT or G tumors results in neither regression of tumor volume nor a cessation of the continuous growth of either tumor. By these criteria, both the AT and G tumors progress following antiandrogen therapy. For the AT tumor, this progression is completely unresponsive to hormonal therapy, and thus such therapy does not increase survival of AT tumor-bearing rats. The AT tumor is therefore an example of hormonally unresponsive progression. In direct contrast, while the G tumor likewise progresses following antiandrogen therapy, this therapy does induce a 1.8-fold decrease in the subsequent growth rate of the G tumor. This positive response during progression of the G tumor results in a 78% increase in the survival of G tumor-bearing rats treated with antiandrogen therapy. The G tumor is therefore an example of hormonally responsive progression.

These results indicate neither that prostatic cancers which do not regress or cease growing following antiandrogen therapy can necessarily be considered hormonally unresponsive nor that antiandrogen therapy of such tumors has been completely ineffective, since, as shown in the present study, such progression can be of either a hormonally unresponsive or a responsive type. Regardless of which type of progression occurs, however, additional therapy is required to further increase survival. The present study demonstrates that such additional therapy should probably not include the subsequent use of pharmacological doses of exogenous androgen, since, depending on the type of progression, such treatments can actually decrease survival.

INTRODUCTION

While prostatic cancers are often responsive to antiandrogen therapy, the extent and duration of such responses are highly variable between individual prostatic cancer patients. Indeed, the National Prostatic Cancer Project has defined and established objective criteria for 4 possible types of clinical response to hormonal therapy (i.e., complete, partial, stable, or progression) (9). By these criteria, patients in whom antiandrogen therapy fails to induce either some regression of established tumor volume or a temporary cessation of tumor growth are considered to be progressing. In such prostatic cancer patients, progression following antiandrogen therapy is usually assumed to mean that the prostatic cancer is hormonally unresponsive and therefore that such therapy was ineffective. This negative interpretation does not consider the possibility that there could be 2 distinct types of progression following antiandrogen therapy (i.e., hormonally unresponsive and responsive progression). Hormonally unresponsive progression could occur when antiandrogen therapy is used to treat prostatic cancers composed of cells which are not dependent nor sensitive to androgenic stimulation in their growth (i.e., androgen-independent insensitive cells). In hormonally unresponsive progression, antiandrogen therapy is ineffective, having no effect upon survival. In contrast, hormonally responsive progression could occur when antiandrogen therapy is used to treat prostatic cancers composed of cells which, while not being dependent upon androgenic stimulation for their growth, are still sensitive to such stimulation (i.e., androgen-independent sensitive cells). Antiandrogen therapy of such an androgen-independent sensitive prostatic cancer would not result in either regression or cessation of the continuous growth of the tumor; however, it could induce a marked decrease in the tumor growth rate. Such a decrease in tumor growth rate would still be considered progression by the present criteria, even though this decrease is a positive response to hormonal therapy. This type of hormonally responsive progression would be distinct, however, from the hormonally unresponsive type of progression, since by inducing a decrease in the tumor growth rate hormonally responsive progression could substantially increase survival.

In order to experimentally determine if in fact there can be 2 distinct types of progression (i.e., hormonally unresponsive progression and hormonally responsive progression), the present study was performed using 2 distinct sublines of the Dunning R-3327 system of serially transplantable rat prostatic adenocarcinomas. The relevance of the Dunning R-3327 system of tumors to human prostatic cancer has been discussed previously (7); based upon these findings, the Dunning R-3327-AT (hereafter called AT) and Dunning R-3327-G (hereafter called G) tumor sublines were chosen as a model system because the AT tumor is composed of androgen-independent insensitive tumor cells (8) while the G tumor is composed of androgen-independent sensitive cells (5). In addition, antiandrogen therapy does not induce either regression of established tumor volume or cessation of the continuous growth of either of these tumors (i.e., both progress following antiandrogen therapy).

MATERIALS AND METHODS

Animals. All animals used in this study were male F. hybrids of the cross between purebred male Copenhagen and female Fischer rats.

Received March 1, 1982; accepted August 23, 1982.

1 This work was supported by Grants CA 15416 and CA 06974 from the NIH, USPHS.

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These F₁ hybrids were inoculated with tumors at 70 to 80 days of age. Castration, when performed, was via the scrotal route using ether anesthesia. Cyproterone acetate (40 mg/kg/day) and DES² (100 µg/kg/day), when given to indicated intact rats, were given as daily s.c. injections in 0.2 ml of propylene glycol.

**Tumors.** Several different fast-growing Dunning R-3327-AT tumor sublines have developed spontaneously from the parent slow-growing R-3327-H rat prostatic adenocarcinoma over the last 6 years in the Brady Laboratories at the Johns Hopkins University. These AT tumors have always been anaplastic in histology and have had a tumor-doubling time of 2 to 3 days. The detailed history and characterization of the particular AT tumor, AT-2, used in the present study have been described previously (6).

The Dunning R-3327-G tumor was originally obtained through the generosity of Dr. Alice Claflin of the University of Miami School of Medicine. This G tumor is histologically a poorly differentiated adenocarcinoma (5). The detailed characterization of this G tumor has been described previously (5). The AT and G tumors were passaged s.c. in the flank of intact male rats by inoculation of $2 \times 10^7$ viable tumor cells/rat as described previously (6).

**Determination of Tumor-doubling Time.** Individual tumor dimensions were serially measured for all tumors using calibrated microcalipers at various times following tumor inoculation. From these measurements, tumor volumes were calculated by the formula $(l \times w \times h) \times 0.5236$. Using the tumor volumes, the tumor-doubling time was determined as described previously (6).

**RESULTS**

**Hormonally Unresponsive Progression following Antiandrogen Therapy of the AT Tumor.** When $2 \times 10^7$ viable AT tumor cells are injected s.c. into the flank of intact adult male F₁ hybrid rats, an AT tumor becomes palpable after 4 to 5 days in essentially 100% of the animals. Once palpable, the growth of these AT tumors is continuous, as demonstrated by the plot of tumor volume versus days post-tumor inoculation (Chart 1). Replotting this growth curve as the logarithm of tumor volume versus days post-inoculation demonstrates that, between 4 and 16 days, the growth of the AT tumor in intact male rats is exponential with a doubling time of 2.8 ± 0.2 (S.E.) days (Chart 1). If intact rats bearing such exponentially growing AT tumors are castrated at Day 10, the tumor does not regress, nor does it cease its continuous exponential growth (i.e., the tumor progresses following castration). In addition, castration has no effect at all on the subsequent growth rate of the AT tumor (Chart 1). These results demonstrate that the AT tumor is completely hormonally unresponsive to castration.

In order to further document the hormonally unresponsive nature of the AT tumor, intact rats bearing AT tumors were separately treated with either of 2 potent antiandrogens, cyproterone acetate or DES. Cyproterone acetate is a potent antiandrogen which functions by inhibiting the translocation of the cytosolic androgen receptor complex into the cell nucleus (3). DES functions as an antiandrogen by lowering the endogenous androgen level via its inhibition of pituitary gonadotrophin secretion (1). Ten days after $2 \times 10^7$ viable AT tumor cells were injected into 15 intact male rats (a time when the AT tumor volumes were 1 to 2 cu cm and were growing with a 2.8-day doubling time), 5 animals were begun on daily cyproterone acetate injections (40 mg/kg/day), 5 animals were begun on daily DES injections (100 µg/kg/day), and 5 animals were given injections of vehicle alone (control). Daily injections were continued until the animals died. During this treatment period, neither cyproterone acetate nor DES induced any regression or even a temporary cessation of the continuous growth of the AT tumor (i.e., the tumors progressed during treatment). In addition, such treatments did not change the tumor-doubling time as compared to that of the control animals (i.e., the doubling times were 2.9 ± 0.1, 2.5 ± 0.2, and 2.7 ± 0.2 days for the cyproterone acetate, DES, and control groups, respectively). These results again demonstrate the complete hormonal unresponsiveness of the AT tumor during progression following antiandrogen therapy (i.e., hormonally unresponsive progression).

**Hormonally Responsive Progression following Antiandrogen Therapy of the G Tumor.** When $2 \times 10^7$ viable G tumor cells are injected s.c. into the flank of intact adult male F₁ hybrid rats, a G tumor becomes palpable after 10 to 15 days in essentially 100% of the animals. Once palpable, the growth of these tumors is continuous, as demonstrated by the plot of the tumor volume versus days post-tumor inoculation (Chart 2). This growth curve can be replotted as the logarithm of tumor volume versus days post-tumor inoculation to demonstrate that between 13 and 32 days the growth of the G tumor in intact male rats is exponential, as shown by the fact that the tumor-doubling time of 4.0 ± 0.2 days is constant during this period (Chart 2). If intact rats bearing such exponentially growing G tumors are castrated at Day 21, the G tumors do not regress in volume, nor do they even temporarily cease growing (i.e.,

² The abbreviation used is: DES, diethylstilbestrol.
they progress). Castration does, however, induce a significant decrease in the growth rate of the G tumor, as reflected by a 1.8-fold increase in the tumor-doubling time from 4.0 ± 0.2 days in the intact to 7.2 ± 0.1 days in the castrated host (Chart 2). The significant decrease in the tumor growth rate, induced by castration, demonstrates that the G tumor does respond positively to antiandrogen therapy.

In order to further document the hormonal responsiveness of the G tumor during its progression following antiandrogen therapy, intact G tumor-bearing male rats were separately treated with cyproterone acetate or DES. Twenty days after 2 × 10^7 viable G tumor cells were injected into 15 intact male rats (a time when the tumor volumes were 1 to 2 cu cm and were growing with a 4-day doubling time), 5 animals were begun on daily cyproterone acetate injections (40 mg/kg/day), 5 animals were begun on daily DES injections (100 μg/kg/day), and 5 were given injections of vehicle alone (control). The daily antiandrogen treatment was continued for the lifetime of the G tumor. Both antiandrogen treatments did, however, decrease by 1.8-fold the growth rate of the tumor to a doubling time of 7.4 ± 0.3 days for the cyproterone acetate group and 7.0 ± 0.2 days for the DES group, as compared to a value of 4.1 ± 0.4 days for the vehicle control group.

These results again demonstrate that the G tumor does respond positively to antiandrogen therapy, even though these tumors progressed following such treatment (i.e., hormonally responsive progression).

Effect on Survival of the Hormonally Responsive Progression of the G Tumor as Compared to the Hormonally Unresponsive Progression of the AT Tumor. Since antiandrogen therapy of established G tumors did affect a positive hormonal response even though progression occurred following therapy, the effects of such a hormonally responsive progression on survival of rats bearing established G tumors were examined.

As a control, the survival of rats bearing established AT tumors was additionally studied, since the hormonally unresponsive progression of these tumors to antiandrogen therapy would not be expected to affect survival. To determine the comparative effect of antiandrogen therapy upon the survival of rats bearing established G versus AT tumors, 20 intact male rats per group were inoculated with either 2 × 10^7 viable G or AT tumor cells. Ten rats per respective group were then castrated when the tumor volumes were 1 to 2 cu cm; this required 20 days for the G tumor group and 10 days for the AT tumor group. All 20 rats (one-half of which were castrated) per respective G and AT tumor group were then allowed to die, and the date of death post-tumor inoculation was recorded for each rat. The time required for 50% of the AT tumor animals to die was identical for both the intact and the castrated AT tumor-bearing rats [i.e., 40 to 45 days (Chart 3)].

No animals survived with the AT tumor for longer than 60 days regardless of androgen status of the host. These results demonstrate that, as expected, the hormonally unresponsive progression of the AT tumor following castration had no effect upon host survival. For intact G tumor-bearing rats not castrated at 20 days postinoculation, the time required for 50% of the G tumor-bearing animals to die was 76 days (Charts 4 and 5). All of these intact G tumor rats were dead by Day 100 (Chart 4). In direct contrast, none of the G tumor-bearing rats which were castrated at 20 days postinoculation, the time required for 50% of the G tumor-bearing animals to die was 76 days (Charts 4 and 5). All of these intact G tumor rats were dead by Day 100 (Chart 4). Instead, the time required for 50% of the castrated rats to die was 135 days, and it took 200 days for all of the rats to die (Charts 4 and 5). These results demonstrate that while castration did not cure any G tumor-bearing rat (i.e., they all progressed), it did prolong survival by 78% (Chart 5). These data demonstrate that castration when tumor volumes are 1 to 2 cu cm produces a hormonally responsive progression in G tumor-bearing rats which results in a substantial increase in host survival.
Hormonally Responsive versus Unresponsive Progression

Effects of Subsequent Treatment with Exogenous Androgen on Survival of Castrated Rats Bearing Progressing AT versus G Tumors. The results of the previous survival studies demonstrate clearly that a progressing prostatic cancer, regardless of whether it is of the hormonally responsive type like the G tumor or of the hormonally unresponsive type like the AT tumor, requires additional therapy in order to further increase survival. Several groups in the past have attempted to treat prostatic cancers progressing following antiandrogen therapy with high doses of exogenous androgen based upon the concept, initially demonstrated with breast cancer, that pharmacological levels of androgen might inhibit tumor growth. The realization that progression following antiandrogen therapy can be either of the hormonally responsive or unresponsive type, however, suggested that such androgen treatment of progressing prostatic cancers might not always be wise. This idea was based upon the concept that, if the progressing prostatic tumor was of the hormonally responsive type, the treatment of such a tumor with exogenous androgen might stimulate its increased growth and thus decrease survival. In contrast, if the progressing tumor was of the hormonally unresponsive type, exogenous androgen treatment would not be expected to decrease but might actually increase survival. In order to test this concept, castrated male rats were inoculated with either 2 x 10^7 viable G or AT tumor cells and allowed to grow without any exogenous androgen treatment. This cycle of passage into castrated male rats unsupplemented with exogenous androgen was then serially repeated 3 more times for both the G and AT tumors. After this fourth serial castrate passage, 10 castrated rats were inoculated with either 2 x 10^7 viable G or AT tumor cells. Tumors appeared in all 10 rats inoculated with either G or AT tumor cells. Five of the castrated rats bearing progressing G tumors and 5 of the castrated rats bearing progressing AT tumors were then begun on a daily pharmacological dose of testosterone (10 mg of testosterone propionate per day) when the respective tumor volumes were 1 to 2 cu cm; this required 40 days for the G tumor group and 10 days for the AT tumor group. All 10 rats (one-half of which were given daily exogenous androgen) per respective G and AT tumor group were then allowed to die, and the date of death postinoculation was recorded for each rat. Treatment of AT tumor-bearing rats with pharmacological doses of testosterone did not affect the time required for either 50 or 100% of the animals to die (Table 1). These results demonstrate that treatment of castrated rats bearing unresponsively progressing AT tumors with a pharmacological dose of androgen has neither a positive nor a negative effect upon survival. In direct contrast, a pharmacological dose of androgen given to castrated rats bearing responsively progressing G tumors does have a profound effect upon survival (Table 1). Such treatment decreased by approximately 40% the survival of such castrated G tumor-bearing rats regardless of whether the time required for 50 or 100% of the animals to die is used for comparison. This decrease in survival of castrated G tumor-bearing rats treated with androgen was found to be directly related to the ability of such androgen treatment to increase the growth rate of the G tumors. In castrated rats not treated with androgen, the tumor, during its exponential phase of growth, grew with a doubling time of 7.5 ± 0.2 days; while in castrated rats treated after Day 40 with androgen, the growth rate increased to a doubling time of only 4.3 ± 0.1 days. These results demonstrate that G tumor cells are not androgen-dependent for proliferation, for if they were, it would have been impossible to serially passage the tumor multiple times in castrated hosts. It is clear, however, that while G tumor cells are not androgen-dependent they are sensitive to androgen stimulation of their growth rate and that this sensitivity can be retained even when the cells are pasaged repeatedly in castrated hosts. These results again demonstrate that the hormonally responsive progression of the G tumor to antiandrogen therapy is distinct from the hormonally unresponsive progression of the AT tumor, since only the hormonally unresponsive progression of the G tumor is capable, following long-term castration, of being stimulated by exogenous androgen treatment.

Table 1

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Treatment of castrated host</th>
<th>Time (days) required for death of</th>
<th>50% of group</th>
<th>100% of group</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>None</td>
<td>40</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Daily testosterone after Day 10a</td>
<td>160</td>
<td>230</td>
<td>100</td>
</tr>
<tr>
<td>G</td>
<td>None</td>
<td>40</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Daily testosterone after Day 40a</td>
<td>160</td>
<td>230</td>
<td>100</td>
</tr>
</tbody>
</table>

a Daily testosterone, 10 mg of testosterone propionate per day.
DISCUSSION

The present studies have demonstrated that when antiandrogen therapy is used to treat prostatic cancers that are composed totally of androgen-independent cells, regardless of whether these are of the completely androgen-insensitive type like the Dunning AT tumor cells or of the androgen-sensitive type like the Dunning G tumor cells, neither regression of established tumor volume nor cessation of the continuous growth of tumor occurs (i.e., the tumor progresses). Such progression is usually assumed to mean that the prostatic cancer was hormonally unresponsive and that therapy was ineffective. This negative interpretation does not consider that there can be 2 distinct types of progression following antiandrogen therapy (i.e., hormonally unresponsive versus hormonally responsive progression) depending upon the specific subtype of androgen-independent cells comprising the prostatic cancer.

If a prostatic cancer is composed of androgen-independent insensitive tumor cells, like the Dunning AT tumor, then antiandrogen therapy of such a tumor is indeed negative, having no effect upon host survival (i.e., hormonally unresponsive progression). In contrast to this well-recognized hormonally unresponsive progression, the present study has demonstrated that if a prostatic cancer is composed of androgen-independent but sensitive tumor cells, like the Dunning G tumor, then antiandrogen therapy, while not inducing either regression or cessation of the continuous growth of the tumor, does induce a substantial decrease in tumor growth rate (i.e., hormonally responsive progression). Such a response is indeed a positive one, since this decrease in tumor growth rate results in a substantial increase in host survival.

The importance of the distinction between hormonally responsive and unresponsive progression following antiandrogen therapy is that it may provide some rationale for several paradoxical clinical observations reported for progressing human prostatic cancers. For example, if all human prostatic cancers progressing after antiandrogen therapy were of the hormonally unresponsive type, this would suggest that the cancer cells comprising the progressing tumor were completely independent of and insensitive to androgenic stimulation for their growth. Therefore, treatment of such hormonally unresponsive progressing prostatic cancers with exogenous androgen should have no stimulatory effect on the tumor. Recently, Fowler and Whitmore (4), however, have reported that in 34 prostatic cancer patients who had progressed following previous antiandrogen therapy, 33 (94%) of these patients had unfavorable responses to subsequent treatment with exogenous testosterone. Such an unfavorable response to exogenous androgen is paradoxical if these progressing tumors were really hormonally unresponsive. In contrast, such an unfavorable response could be explainable if those progressing tumors were actually hormonally responsive, being composed of androgen-independent but sensitive tumor cells. This is because, as demonstrated with the G tumor, these androgen-independent sensitive cells can retain their androgenic sensitivity even after prolonged periods without androgen.

A second point to which the distinction between hormonally responsive and unresponsive progression may be very important relates to the use of the androgen receptor content of human prostatic cancers to predict their subsequent response to antiandrogen therapy. While several human studies have demonstrated that there is a fairly good correlation between the androgen receptor content and the subsequent positive response of individual prostatic cancer patients to antiandrogen therapy, such studies have additionally demonstrated that there are a substantial number of patients who still progress to hormonal therapy despite having androgen receptor-positive tumors (2, 10). The paradoxical progression of these androgen receptor-positive tumors has led some investigators to doubt the value of androgen receptor content as a predictive indicator. The realization that progression of prostatic cancer to antiandrogen therapy can be of either a hormonally responsive or unresponsive type and that hormonally responsive progression is in fact a positive response, however, may resolve much of this paradox. If a prostatic cancer was composed of androgen-independent but sensitive tumor cells, then such sensitive cells would be expected to be androgen receptor positive. Antiandrogen therapy of patients with such an androgen-independent sensitive prostatic cancer would result in progression even though this androgen receptor-positive tumor responded positively to hormonal therapy with a decrease in its growth rate (i.e., hormonally responsive progression). Such a hormonally responsive progression would not be paradoxical, however, since androgen receptor content predicts only whether a response will occur; it does not predict the exact type of response (i.e., complete, partial, stable, or hormonally responsive progression) to be expected.

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