Treatment of R-3327 Prostate Tumors with a Somatostatin Analogue (Somatuline) as Adjuvant Therapy following Surgical Castration

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ABSTRACT

This study addresses, in an animal tumor model, the clinical problem of "escape from castration inhibition." Somatuline (BIIY1-23014C), an octapeptide analogue of somatostatin with enhanced potency and longer duration of biological activity was administered as a therapeutic agent, over a period of 90 and 197 days, to male Copenhagen rats bearing syngeneic Dunning R-3327-H prostate tumors. Androgen sensitivity was confirmed by the response of tumors to castration and by the significant inhibition of tumor growth in intact animals by treatment with a lutetinizing hormone-releasing hormone antagonist (BIM-21009). Inhibition of tumor growth resulting from castration persisted for 102 days, after which progressive regrowth occurred, indicating an escape from castration inhibition. When Somatuline treatment was initiated as an adjuvant therapy 5 days after castration, the rate of tumor regrowth during escape was significantly retarded. During the period of 197 days postcastration, tumors in the vehicle-treated, intact controls grew to an average diameter of 38.6 ± 7.6 mm and tumors in vehicle-treated castrate controls grew to an average diameter of 23.3 ± 4.1 mm (60% test/control). Treatment with the lutetinizing hormone-releasing hormone antagonist induced no significant additional tumor inhibitory effects in castrated animals which developed tumors having an average diameter of 30.2 ± 8.2 mm (78% test/control). Treatment of tumors in castrate animals with Somatuline, on the other hand, produced a significant (P < 0.01) tumor-inhibitory effect that was greater than that produced by castration alone, developing an average tumor diameter of only 14.3 ± 2.6 mm, (37% test/control). A growth inhibitory effect was also inducible in animals having tumors that had already escaped castration inhibition. The relative nontoxicity of a somatostatin analogue such as Somatuline suggests that chronic or maintenance therapy of slow-growing prostate cancers may be both feasible and acceptable in a clinical setting.

INTRODUCTION

Prostatic carcinoma is the most common malignancy in elderly men, accounting for 25,500 annual deaths in the United States (1). Treatment of symptomatic advanced prostate cancer by orchidectomy or estrogen administration controls symptoms in 70–80% of patients, and has been reported to improve 5-year survival from approximately 5 to 25% (2). Hormone deprivation or surgical ablation to control prostatic carcinoma, although leading to a period of remission, is invariably followed by a relapse of the disease (3, 4). Once progression occurs on hormonal therapy, no common opinion exists on further treatment. The fate of the patient with evidence of tumor progression after an initial attempt at hormonal therapy is grim, with a mean survival less than 1 year (5).

Prostatic adenocarcinoma in humans is characterized by diversity in relation to pathology, state and variability of cellular differentiation, uniformity of growth rate, and differences in therapeutic responsiveness to hormonal, nonhormonal, and radiation treatment. Although such diversity is more commonly observed between individuals, many of these variations can be observed within one patient.

Relevancy of the Dunning R-3327-H prostatic adenocarcinoma of the rat (6) as a model of the human disease has been demonstrated by the number of distinct sublines established in serial transplantation that show distinct differences with respect to histology, growth rate, metastatic activity, and androgen sensitivity (7–11). The basic heterogeneity of this tumor system, in terms of its composition as a heterogeneous mixture of preexisting clones of both androgen dependent and independent tumor cells, permits mimicking the clinical phenomenon of "escape from castration" or the relapse from an initial positive response.

Recent advances in growth factor and oncogene research support the hypothesis of autocrine secretions by neoplasms, i.e., cancer cells can produce and respond to their own growth factors (12), suggesting the potential therapeutic effectiveness of growth inhibitors such as somatostatin or its analogues. The results of current intensive research into the nature of oncogenes such as src and the biology of growth factors such as epidermal growth factor indicate that certain cellular components utilized by these factors are, in an altered form, encoded by certain viral oncogenes (13–15).

Since cellular transformation and the normal biological actions of growth factors are functionally related by their apparent utilization of common, but specific, cellular biochemical processes, it would suggest that transformed cells may have acquired a pleiotropism for responsiveness to normal growth factors. The apparent functional homology with the tyrosine kinase family of oncogenes and the known sequence of the epidermal growth factor receptor (12), for example, would further suggest that the common biochemical pathways may not only have an increased sensitivity to growth stimulators, but may have retained their sensitivity for the growth inhibitors.

If, as postulated by Wahl and Carpenter (16), that the normal proliferation involves both autocrine and paracrine stimulatory phenomena and that normal control of growth involves the differential response to tonic concentrations of several growth-regulating hormones at once, then changes in the relative concentrations of these regulators might induce dramatic alterations in cell morphology, physiology, and growth. Increased secretions of autocrine growth factors favor the growth autonomy of neoplasms, but do not necessarily abrogate their responsiveness to the growth inhibitors such as SRIF and its analogues. The findings that somatomedins may act by paracrine or autocrine mechanisms (17) and that concentrations of somatomedin C in tissues are growth hormone dependent (18) would suggest that cancers should be responsive to the more potent SRIF and to SRIF when administered at concentrations exceeding normally attainable physiological levels (19).

Lamberts et al. (20), Klijn et al. (21), and Moreau and deFeudis (22) have reviewed the potential role of somatostatin analogues in the treatment of cancer. Growth-inhibitory effects have been obtained in vivo against a limited number of human...
and animal tumors. Of particular importance are the demonstrations of antiproliferative effects on cancer cells in vitro (23–27), indicating that somatostatin and analogues can act directly on cancer cells.

The preceding experimental observations and resultant hypotheses suggest that antiproliferative agents such as somatostatin and its analogues should show antitumor activity when tested against androgen-responsive as well as androgen-unresponsive tumors in intact (noncastrated) as well as castrated hosts. Since doses higher than the normally attainable physiological concentrations may be required to compete successfully with paracrine or autocrine growth factors at the tumor site, somatostatin analogues should also be sufficiently devoid of undesirable side effects to permit the administration of clinically effective therapeutic doses. The feasibility of delaying the escape from castration inhibition by treating prostate tumors with a somatostatin analogue as adjuvant therapy following surgical castration is addressed in this study.

MATERIALS AND METHODS

The Dunning adenocarcinoma of the rat prostate, designated R-3327-H, was provided by Norman Altman (Papanicolaou Cancer Research Institute, Miami, FL) through the courtesy of the Prostate Cancer Working Group, Organ Systems Coordinating Center, National Cancer Institute. It is slow growing, androgen responsive, and propagated in Copenhagen × Fischer F1 males. For these studies, the tumor was implanted and tested in syngeneic Copenhagen strain males. Test and control animals were implanted s.c. in the right flank with a 3- to 5-mm3 mince of prostate tumor tissue. Resultant tumors were measured weekly with Vernier calipers and tumor size was calculated as the average of the longest and shortest diameters (length + width/2) mm. Levels of significance were determined with Student's t test.

Testes were surgically removed (castration) under ether anesthesia. Orchidectomy 15 to 20 days posttumor implantation was considered “early castration” and orchidectomy during logarithmic phase of growth (days 129 to 132) was considered as “late castration.”

Male Copenhagen rats were obtained from the Biological Testing Branch, Frederick Cancer Research Facility, Division of Cancer Treatment, National Cancer Institute. After a 2-week period for acclimatization, animals were weighed individually, identified by ear markings, and then randomized into the various control and test groups.

First Study. To test the responsiveness of the R-3327-H rat prostate tumor to Somatuline in animals with intact testes, 34 male Copenhagen rats were implanted s.c. with R-3327-H tumor tissue and treated as shown in Table 1.

Second Study. To test the effectiveness of Somatuline on growth of R-3327-H prostate tumors escaping castration inhibition, 42 male Copenhagen rats were implanted s.c. with R-3327-H tumor tissue and treated as follows.

Group 1, saline vehicle-treated controls with intact testes (10 rats), received 0.2 ml/injection, s.c., twice daily, on days 15–334; Group 2, castrated day 15, saline vehicle-treated controls (10 rats), received 0.2 ml/injection, s.c., twice daily, on days 15–334; Group 3, castrated day 137, saline vehicle-treated controls (10 rats), received 0.2 ml/injection, s.c., twice daily, on days 137–334; Group 4, castrated day 132 (6 rats), received an LH-RH antagonist (BIM-21009), 500 μg/injection, s.c., on days 137–334; and Group 5, castrated day 132 (6 rats), received Somatuline, 250 μg/injection, s.c., twice daily, on days 137–334.

Somatuline (BIM-23014C, DC13-116), an endocrinologically potent octapeptide analogue of somatostatin

D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH₂ acetate was administered s.c. at 50 μg/injection (low dose) or at 250 μg/injection (high dose), twice daily, in a saline vehicle (26, 27). BIM-21009, an antagonist of LH-RH [NAc-D-Nal-D-Pro-D-Phe-D-Phe-Ser-Tyr-D-Arg-Phe-Arg-Pro-D-Ala-NH₂ acetate, was administered s.c. in a saline vehicle at 500 μg/injection, once daily.

RESULTS

First Study. Androgen sensitivity of the R-3327-H prostate tumor used in this study is illustrated by the response to castration on day 129 posttumor implantation (Fig. 1). To determine the responsiveness of well-established R-3327-H prostate tumors to Somatuline, treatment was not initiated until day 130 posttumor implantation (Fig. 1). Average tumor diameter at that time was 10.3 mm. A control tumor-bearing group was castrated on day 129. The study was terminated after 90 days of treatment. Final tumor weights are shown in Table 1. Both castration and Somatuline treatment induced a significant and similar retardation of tumor growth. Tumor growth curves illustrated in Fig. 1 show that the deceleration of tumor growth rate was similar in castrates and intact animals treated with Somatuline. A plateauing of the Somatuline-treated tumor growth curve with time suggests a resultant induction of "stable disease," a condition that would be expected to extend survival time.

Organ weight responses in intact animals bearing R-3327-H prostate tumors and treated with Somatuline are compared with organ weight responses in tumor-bearing castrate animals (Table 2). In addition to the significant inhibition of prostate tumor growth resulting from castration (Table 1), there was significant atrophy of the ventral prostate. By contrast, although Somatuline treatment induced an inhibition of prostate tumor growth equivalent to that of castration (Table 2), there was no significant atrophy of the ventral or whole prostate and testes. The apparent absence of antiandrogenic activity confirms the absence of an effect on testicular weight and plasma testosterone levels observed in an earlier study (28).

Second Study. To determine the effect of Somatuline on growth of tumors escaping castration inhibition, male Copenhagen rats, approximately 90 days old, were implanted s.c. with R-3327-H tumor tissue and then groups were castrated on day 15 posttumor implantation or on day 132 when tumors were well established, having average diameters of 16.7 ± 3.6 mm.

The relatively effective efficacy of early castration (day 15) as compared to late castration (day 137) is illustrated by the tumor growth curves in Fig. 2 (Groups 2 and 3), confirming Isaac's initial demonstration (29) that the time of castration does have a direct effect upon the maximum therapeutic benefit to be derived from such therapy.

The continuing androgen sensitivity of this in vivo passage of the R-3327-H tumor was also confirmed.

Group 4 animals castrated on day 132 were treated with the LH-RH antagonist, whereas Group 5 animals castrated on day 132 were treated with Somatuline. Treatment was initiated 5 days after castration and continued over a period of 197 days. Castration inhibition of tumor growth was evident within a measurement period of 7-10 days with an eventual plateauing effect in all castrate groups. Inhibition of tumor growth resulting from castration persisted for approximately 102 days. Duration of castration inhibition was similar in treated as well as in the untreated castrates.

Once escape from castration inhibition was evident, however, tumors in all groups grew progressively (Fig. 2). From castration day 132 posttumor implantation (Fig. 2), tumors in the vehicle-treated, intact control grew an average diameter of 38.6 ± 7.6 mm and in the vehicle-treated castrated controls, 23.3 ± 4.1 mm (60% test/control). Treatment of castrated animals...
RESPONSE OF ANDROGEN INSSENSITIVE PROSTATE TUMORS TO SOMATULINE

![Fig. 1. Growth curves of R-3327-H prostate tumors in intact and castrate animals and in intact animals treated with Somatuline.](image)

Table 1 Response of R-3327-H tumors to somatuline in Copenhagen strain rats with intact testes

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Tumor wt (g) day 220</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact, vehicle control, 0.2 ml/injection, s.c., b.i.d.</td>
<td>13.8 ± 4.4</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>Castrate, vehicle control, 0.2 ml/injection, s.c., b.i.d., q.d. 130-220</td>
<td>2.6 ± 0.9</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Intact, Somatuline, 50 µg/injection, s.c., b.i.d., q.d. 130-220 (castration day 129)</td>
<td>3.0 ± 1.4</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>Intact, Somatuline, 10 µg/injection, s.c., b.i.d., q.d. 130-220</td>
<td>3.1 ± 2.7</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>Intact, Somatuline, 2 µg/injection, s.c., b.i.d., q.d. 130-220</td>
<td>5.5 ± 1.4</td>
<td>5</td>
</tr>
</tbody>
</table>

* Tumor weight presented as means ± SEM.
* b.i.d., twice daily; q.d., every day.
* Significance of difference from intact control, P < 0.05.

With only three tumors involved, the response of each tumor to Somatuline was examined over a period of 28 days, i.e., 14 days before Somatuline treatment and 14 days after treatment was initiated. Group response (average of three tumors) showed a growth of 2.4 mm during the period prior to treatment and essentially no growth (—0.2 mm), after initiation of treatment, i.e., stable disease. When examined individually, Somatuline treatment induced a therapeutic antitumor effect in all three animals that had escaped castration inhibition, as evidenced by the parameters of growth inhibition, stable disease, and a partial remission (Table 3).

**DISCUSSION**

The androgen sensitivity and basic heterogeneity of the Dunning R-3327-H adenocarcinoma of the rat prostate, in terms of its composition as a heterogeneous mixture of preexisting clones of both androgen-dependent and -independent tumor cells, was confirmed by its responsiveness to castration and the phenomenon of escape from castration inhibition. Responsive-ness of R-3327-H prostate tumors in the intact animal to somatostatin analogues (28, 30, 31) was also confirmed in this study. Of particular clinical relevance was the demonstration of antitumor activity in the castrate animal, suggesting a therapeutic efficacy in treating prostate tumors with Somatuline as adjuvant therapy following surgical or chemical ablation.

Immunoreactive somatostatin has been detected in the prostate, testis, epididymis, and seminal vesicles of male rats (32) and humans (33). The physiological significance of somatostatin in the reproductive system and the mechanisms of its...
RESPONSE OF ANDROGEN INSSENSITIVE PROSTATE TUMORS TO SOMATULINE

Table 2 Organ weight responses in animals bearing the R-3327 prostate tumor and treated with Somatuline, a somatostatin analogue

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Organ wt'</th>
<th>T/C</th>
<th>T/C</th>
<th>T/C</th>
<th>T/C</th>
<th>T/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Intact, saline control, 0.2 ml/injection, q.d. 130-220, b.i.d., s.c.</td>
<td>6.89 ± 0.54</td>
<td>30.55 ± 5.12</td>
<td>2.02 ± 0.64</td>
<td>46.29 ± 14.50</td>
<td>210.49 ± 67.37</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Castrate, saline control, 0.2 ml/injection, q.d., 130-220, b.i.d., s.c.</td>
<td>7.94 ± 1.46</td>
<td>115</td>
<td>28.89 ± 4.24</td>
<td>95</td>
<td>8.11 ± 3.11</td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>Intact, Somatuline, 50 µg/injection, q.d. 130-220, b.i.d., s.c.</td>
<td>6.52 ± 0.57</td>
<td>95</td>
<td>29.06 ± 2.45</td>
<td>95</td>
<td>2.00 ± 0.28</td>
<td>99</td>
</tr>
<tr>
<td>4</td>
<td>Intact, Somatuline, 10 µg/injection, q.d., 130-220, b.i.d., s.c.</td>
<td>6.37 ± 0.73</td>
<td>92</td>
<td>27.82 ± 2.89</td>
<td>91</td>
<td>2.12 ± 0.13</td>
<td>105</td>
</tr>
<tr>
<td>5</td>
<td>Intact, Somatuline, 2 µg/injection, q.d. 130-220, b.i.d., s.c.</td>
<td>6.35 ± 0.52</td>
<td>92</td>
<td>27.25 ± 2.95</td>
<td>89</td>
<td>2.08 ± 0.14</td>
<td>103</td>
</tr>
</tbody>
</table>

* Male Copenhagen rats used for this study, 5 to 9 animals/group.
* Compounds were prepared fresh every week, daily aliquots made and stored in a freezer until needed for injection.

Fig. 2. Response of R-3327-H prostate tumors to early group (Gr.) 2 and late group (Gr.) 3 castration. Group 3 illustrates escape of R-3327-H prostate tumors from castration inhibition, and group 5 the escape-inhibiting effect of Somatuline treatment. Treatment of prostate tumors escaping castration inhibition with an LH-RH antagonist (group 4) had no therapeutic effect.

Table 3 Effect of Somatuline on growth of tumors that have escaped castration inhibition

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Tumor growth before treatment</th>
<th>Tumor growth after treatment</th>
<th>Antitumor effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0 mm</td>
<td>1.5 mm</td>
<td>Growth inhibition</td>
</tr>
<tr>
<td>14</td>
<td>3.0 mm</td>
<td>-0.5 mm</td>
<td>Stable disease</td>
</tr>
<tr>
<td>54</td>
<td>1.0 mm</td>
<td>-1.5 mm</td>
<td>Partial remission</td>
</tr>
</tbody>
</table>

* Tumor size measured over a 28-day period, i.e., 14 days before Somatuline treatment and 14 days after treatment was initiated, reported as change in tumor size during each 14-day period. A negative number indicates tumor regression.

**Tumor synthesis and release are not known. However, its ubiquity and broad spectrum of biological activity (34, 35) indicate somatostatin to be an endogenous regulatory growth inhibitor (31, 34, 36) and suggests that Somatuline may have inhibited prostate tumor growth in intact and castrated males by interfering with the secretion of paracrine, autocrine, as well as endocrine growth factors.

In contrast to the classical cytotoxic chemotherapeutic agents which exert a therapeutic oncolytic effect by inducing cell death directly, antiproliferative agents such as somatostatin elicit a therapeutic response by depriving tumor cells of growth factors. Thus, the tumor growth inhibition obtained with the antiproliferative peptides is more slowly evolving in contrast to the dramatic oncolytic effects (marked tumor regressions) that are induced with cytotoxic chemotherapeutic agents, an observation suggesting that early stabilization of disease is a positive therapeutic response.
able, especially in prostate cancer, if this state of therapeutic response can be achieved with minimal or no systemic toxicity and results not only in prolongation of survival, but in a palliation that enhances the quality of life. Increasing the non-proliferating cell population may, in time, also increase the rate in number of cells reaching senescence and dying with a resultant therapeutic response of partial remission or complete remission.

Because of their relatively direct modes of action, cytotoxic chemotherapeutic agents such as the nitrogen mustards tend to be more effective against the fast-growing, high mitotic index neoplasms. On the other hand, antiproliferative peptides such as somatostatin analogues should be most effective against the slow-growing (low mitotic index) neoplasms. The in vivo responsiveness of a number of human and animal tumors having a spectrum of histological types and growth rates to Somatuline would support this conclusion (19). An equally important clinical consideration is the relative nontoxicity of somatostatin analogues, suggesting that chronic or maintenance therapy may be both feasible and acceptable.

REFERENCES

Treatment of R-3327 Prostate Tumors with a Somatostatin Analogue (Somatuline) as Adjuvant Therapy following Surgical Castration


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