ABSTRACT

Receptors for somatostatin (SST) that are found on prostate cancers might be used for targeting of chemotherapeutic agents. Thus, doxorubicin derivative 2-pyrrolinodoxorubicin (AN-201) can be linked to SST analogue RC-121 (o-Phe-Cys-Tyr-d-Trp-Lys-Val-Cys-Thr-NH₂) to form targeted cytotoxic SST analogue AN-238. In this study, we evaluated the effects of AN-238 on the growth of SST receptor (SSTR)-positive androgen-independent Dunning R-3327-AT-1 prostate cancers in rats. The dose range and tumor growth-inhibitory effects of AN-238 and AN-201 were investigated in preliminary experiments. Administration of cytotoxic radical AN-201 at single i.v. doses of 110, 125, and 150 nmol/kg resulted in 0, 77.7, and 100% mortality, respectively, within 6–10 days. Four weeks after the injection of 110 nmol/kg AN-201, mean tumor volume was reduced by 35.1% (P < 0.05), as compared with controls. In contrast, a single i.v. injection of analogue AN-238 at a dose of 300 nmol/kg was nontoxic and remarkably potent in inhibiting the growth of Dunning AT-1 tumors, resulting in a 85.9% (P < 0.01) reduction in tumor volume after 4 weeks. Treatment with AN-238 extended the survival time of tumor-bearing rats from 52.0 ± 3.75 to 91.8 ± 3.70 days, corresponding to a 76.5% (P < 0.01) increase. In a comprehensive experiment, we compared the effects of radical AN-201 at 115 nmol/kg, analogue AN-238 at 115 and 300 nmol/kg, carrier SST analogue RC-121 at 300 nmol/kg, and a mixture of AN-201 and RC-121 at doses of 300 nmol/kg administered i.v. Administration of AN-201 at 115 nmol/kg led to 90.0% mortality in 12 days, but animals treated with 115 nmol/kg of AN-238 showed no signs of toxicity, their tumor volume was reduced by 48.8% (P < 0.01) after 4 weeks, as compared with controls. The dose of 300 nmol/kg of AN-238 was also nontoxic and diminished tumor volume by 80.9% (P < 0.01) and tumor weight by 82.0% (P < 0.01). No reduction in tumor growth or toxic effects was observed with carrier RC-121, but after the injection of unconjugated mixture of AN-201 and RC-121 at doses of 300 nmol/kg, all rats died within 4 days. Specific high-affinity receptors for SST were found on Dunning R-3327-AT-1 tumor membranes by radioligand binding assay and were identified by reverse transcription-PCR as SSTR2. Our study indicates that cytotoxic SST analogue AN-238 can be targeted to SSTRs on tumors and produces a powerful inhibition of the growth of Dunning-AT-1 rat prostate cancer at doses that are nontoxic, whereas its cytotoxic component, 2-pyrrolinodoxorubicin, is toxic and ineffective.

INTRODUCTION

Carcinoma of the prostate is the most common malignant tumor in men, and in the United States, ~185,000 new cases and ~39,000 deaths from this disease are estimated for 1998 (1). About 40% of new diagnoses are made at an advanced stage of the disease, when cure by radical prostatectomy is not possible (2). The current therapeutic methods for the management of advanced prostate cancer are palliative and based upon androgen deprivation (3–5). However, castration, androgen-deprivation, or analogues of luteinizing hormone-releasing hormone can only provide a remission (4), and most patients will relapse from androgen control in 18–36 months (3–5). Therapeutic options are limited for patients who relapse. The treatment of androgen-independent prostate cancer is a major oncological challenge, and new therapeutic approaches must be developed. Low response rates with high toxicity are found with conventional chemotherapy (6). Weekly administration of doxorubicin, which is one of the agents used in the therapy of advanced androgen-independent prostate cancer, produces a weak response with significant toxic effects (7). A local delivery of chemotherapeutic agents to malignant cells would greatly reduce their toxicity while enhancing the tumoricidal effect (3). Thus, targeted cytotoxic agents could be formed by linking various cytotoxic radicals to peptide hormone carriers that have specific binding sites on tumor tissues (3).

Various primary human tumors, such as endocrine, mammary, gastric, renal, and prostatic tumors, and tumor cell lines were shown to possess SSTRs (3, 8–14). Experimental oncological studies demonstrated that SST and its octapeptide analogues, such as octreotide or RC-160, can inhibit the growth of some of these malignancies (3, 15, 16). Most of the octapeptide SST analogues bind selectively to SSTR2 and SSTR5 subtypes (17). Although SSTR2 subtype was not found in primary prostate cancer specimens using in situ hybridization, the expression of octreotide-prefering SSTRs on metastatic hormone-refractory prostatic adenocarcinoma has been verified (18, 19). In addition, the expression of SSTR5 subtype in epithelial cells from primary cultures of surgically removed prostate cancer specimens was demonstrated by the RT-PCR method (9). High-affinity binding of octapeptides RC-160 and RC-121 was also described on human prostate cancer specimens removed by radical prostatectomy (14). Tumor growth inhibition and direct antiproliferative effect of SST or its analogues have also been demonstrated in rat prostate cancer models, in human prostatic cancer cell lines xenografted into nude mice, and in vitro (11, 20–24). Clinical studies have demonstrated improvement in performance status and bone pain, fall in PSA, and prolongation of survival in patients with metastatic hormone-refractory prostate cancer after treatment with some SST analogues (25, 26).

The demonstration of SSTRs on various cancers stimulated the development of radiolabeled octapeptide analogues for the localization of tumors (3, 16, 27, 28). Thus, [111In-DTPA-D-Phe₆]octreotide was successfully used for scintigraphic imaging of different cancers in >1000 patients (29). These findings and our previous experience with the antitumor effects of an early targeted cytotoxic SST analogue, AN-51, consisting of RC-121 and methotrexate (30), prompted us to develop modern, targeted cytotoxic analogues of SST. Recently, we described the synthesis and evaluation of such SST analogues con-

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4 The abbreviations used are: SSTR, somatostatin receptor; SST, somatostatin; RT-PCR, reverse transcription-PCR.
taining doxorubicin or its intensely potent derivative 2-pyrrolinodoxorubicin (31). These compounds were developed for therapy of cancers that possess receptors for SST (31).

Here, we evaluated the antiproliferative effects of targeted cytotoxic SST analogue AN-238 in rats bearing the rapidly growing and hormone-insensitive Dunning R-3327-AT-1 prostate cancer, which was previously shown to bind SST octapeptides such as RC-160 (24). The effects of analogue AN-238 were compared to those exerted by its components, carrier SST analogue RC-121 and cytotoxic radical AN-201.

MATERIALS AND METHODS

Peptides and Cytotoxic Agent. The SST analogue RC-121 (p-Phe-Cys-Tyr-d-Trp-Lys-Val-Cys-Thr-NH2) and 2-pyrrolinodoxorubicin (AN-201) were synthesized in our laboratory as described (32, 33). The cytotoxic conjugate AN-238 was made by coupling one molecule of 2-pyrrolinodoxorubicin-14-O-hemiglutarate to the NH2 terminus of [(Lys(4-N-fluorenyl)-methoxycarbonyl)Y]JRC-121, followed by deprotection and purification (31). The SST analogue RC-160 was synthesized by Debiopharm (Lausanne, Switzerland). Before i.v. injection, all compounds were dissolved in 20 μL of 0.1 N acetic acid and diluted with 5% (w/v) aqueous d-mannitol solution (Sigma Chemical Co., St. Louis, MO).

Animals. Six-week-old male Copenhagen rats were purchased from the National Cancer Institute (Frederick Cancer Research and Development Center, Frederick, MD). The rats were housed four per cage in a temperature-controlled room with a 12-h light/12-h dark schedule and were given water and standard rat Chow ad libitum. All studies were performed in accordance with institutional guidelines of animal care and the welfare of animals in experimental neoplasia.

Experimental Protocols. The Dunning R-3327-AT-1 tumor cells were originally kindly provided by Dr. John T. Isaacs (Department of Urology, James Buchanan Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, MD) and maintained in tissue culture as described (24). Dunning R-3327-AT-1 tumor cells (3 × 105) were injected i.c. into three rats. After 2 weeks, resulting tumors were aseptically dissected and mechanically minced; 3-mm3 pieces of tumor tissue were transplanted s.c. by trocar needle into rats under methoxyflurane anesthesia (Metofane; Pittman-Moore, Mundelein, IL). The experiments started when tumors had grown to 70 mm3 in volume. In the first experiment, single i.v. injections of AN-201 at 110, 125, and 150 nmol/kg doses were given to rats (six to nine per group) through the jugular vein under anesthesia. Tumor growth was measured weekly using microliter-pipettes. Body weights and deaths related to toxicity were recorded. In a second experiment, groups of five rats were injected i.v. with either 300 nmol/kg of AN-238 or vehicle (controls). Tumor volumes and body weights were measured weekly, and survival time in both groups was determined. In a third, comprehensive experiment, six groups of rats were used. The first group, composed of 10 rats, received an i.v. injection of AN-201 at 115 nmol/kg. The second and third groups received AN-238 at 115 nmol/kg (seven rats) and 300 nmol/kg (eight rats). Other groups were injected with the mixture of AN-201 and carrier SST analogue RC-121 at the doses of 300 nmol/kg (four rats) or carrier SST analogue RC-121 at 300 nmol/kg (five rats). The control group, consisting of eight rats, was given the injection vehicle. This experiment lasted for 4 weeks, and tumor volumes were recorded weekly. Blood samples were collected once a week from the tail vein with Unopette microcollection kit (Becton Dickinson, Franklin Lakes, NJ). WBC and platelet counts were determined manually using a hemacytometer. The changes in WBC and platelet count were evaluated on the basis of results previously reported in rats (34), and values out of that normal range were expressed as a percentage of the corresponding value found in controls. The toxicity was evaluated on the basis of changes in WBC and platelet counts and body weights of animals. Tumor weight was measured at autopsy, and tumor burden was calculated (mg of tumor weight/g of body weight). Samples of each tumor were snap-frozen and stored at −70°C for preparation of membranes for receptor assay and for extraction of RNA for RT-PCR.

Receptor Assay. Binding of octapeptide RC-160 to SSTR on tumor membranes preparations was determined by ligand competition assays using 125I-labeled RC-160, as reported previously (31). The LIGAND PC computerized curve-fitting program of Munson and Rodbard (35) was used to determine the type of receptor binding, the dissociation constant (Kd), and the maximal binding capacity (Bmax) of (receptors (35). Receptor binding affinity of cytotoxic SST analogue AN-238 to tumor membranes was measured in displacement experiments based on competitive inhibition of 125I-RC-160 binding using various concentrations of AN-238 (10−16–10−12 M). IC50, defined as the dose causing 50% inhibition of 125I-RC-160 binding, was calculated by a computerized curve-fitting program (36).

RT-PCR. The expression of SSTR2 and SSTR5 subtypes and β-actin (internal control) in tumor tissue was assessed. Total RNA was extracted using RNAzol B (Tel-Test, Inc., Friendswood, TX) and quantified by spectrophotometer at 260 nm. All reagents used for RT-PCR were purchased from Perkin-Elmer (Norwalk, CT) unless otherwise specified. To avoid genomic DNA contamination, all samples were subjected to DNase treatment before RT-PCR. RNA (500 ng) was treated with 0.3 unit of RNase-free DNase (Promega, Madison, WI) in a total reaction volume of 19 μL containing 5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM each dNTP, 1 unit/μL RNase inhibitor, and 2.5 μM random hexamers at 37°C for 30 min. After denaturation at 99°C for 5 min, 1 μL of 50 units/μL murine leukemia virus reverse transcriptase was added, and reverse transcription was carried out for 1 h at 42°C and terminated by denaturation of the enzyme at 99°C for 5 min. Negative controls were run, with the addition of 1 μl of diethyl pyrocarbonate-treated water instead of murine leukemia virus reverse transcriptase.

Five μl of the reverse transcribed products were amplified in a 25-μl reaction volume containing 1.5 mM MgCl2, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.2 mM each dNTP, 0.12 μM each sense and antisense primer, and 0.3 μl of 5 units/μl AmpliTaq DNA polymerase. In accordance with the published sequences (37, 38) and a previous study (39), the following primers were used: SSTR2 sense, 5'-AGAAGGACTGATGGATGTTGACCTCTG; SSTR2 antisense, 5'-CATCTGGCCATGGCGACCTGTT; SSTR3 sense, 5'-GACCTGAGCATGAGCCCCTTC; SSTR3 antisense, 5'-GTGCAGCCACGCAGCGCTTT; β-actin sense, 5'-GTTCACCCCACTGCTGCCATCT; and β-actin antisense, 5'-ACAGACTATCTGGCTCAGGAG. The samples were denatured for 2 min at 94°C and then amplified in Robocycler (Stratagene, La Jolla, CA) for 30 cycles (SSTR2), 40 cycles (SSTR5), or 20 cycles (β-actin) with a step program of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min. After a final step of 72°C for 10 min, 5 μL of the PCR samples were subjected to electrophoresis in 8% polyacrylamide gel and stained by silver. As a reference, brain tissue from a normal rat was used.

Statistical Analysis. Statistical analyses of the data were performed using Duncan’s multiple range test (40). Data were expressed as mean ± SE.

RESULTS

Toxicity and Tumor Growth Inhibition. In the first experiment, different i.v. doses of cytotoxic radical AN-201 were given to rats bearing Dunning R-3327-AT-1 tumors. AN-201 at 110 nmol/kg reduced the body weight by 11.8% (P < 0.05), 1 week after injection, as compared with controls, but no deaths occurred in this group. However, 77.7% and 100% mortality occurred within 6–10 days, after administration of 125 and 150 nmol/kg of the cytotoxic radical, respectively (Fig. 1). All deaths were preceded by significant loss in body weight. Four weeks after injection of 110 nmol/kg of AN-201, the tumor volume was reduced by 35.1% (P < 0.05), but there was no significant decrease in tumor weight, as compared with controls.

In the second experiment, a single injection of cytotoxic SST analogue AN-238 at a dose of 300 nmol/kg had no significant effect on the body weight and no deaths occurred due to toxicity (Fig. 1), but 4 weeks later, an 85.9% (P < 0.01) decrease in tumor volume was found, as compared with controls. AN-238 also extended the mean survival time of tumor-bearing rats from 52.0 ± 3.75 to 91.8 ± 3.70 days (P < 0.01), corresponding to a 76.5% increase.

The third comprehensive experiment was designed to compare the antiproliferative effects and toxicity of single i.v. injections of radical AN-201, hybrid analogue AN-238, carrier SST analogue RC-121, and
The unconjugated mixture of AN-201 and RC-121. AN-201, injected at 115 nmol/kg, resulted in a 32.2% (P < 0.01) weight loss, accompanied by a 90.6% (P < 0.01) decrease in WBCs during the first week. In some of the rats, diarrhea occurred, and four of eight rats had thrombocytopenia. Because of toxicity of AN-201, 9 of 10 animals died within 12 days (Fig. 1). Analogue AN-238, given at 115 nmol/kg, did not affect the body weight or WBC and platelet count, and none of the rats died (Fig. 1). After 4 weeks, when the experiment ended, in the group treated with 115 nmol/kg of AN-238, the tumor size was reduced by 40.0% (P < 0.05), tumor weight was reduced by 42.8% (P < 0.01), and tumor burden was reduced by 37.0% (P < 0.05), as compared with controls (Fig. 2 and Table 1). Rats that received 300 nmol/kg of analogue AN-238 gained somewhat less weight than the controls during the first week of the experiment, and 7 days after the injection, the average body weight was 10.5% (P < 0.01) lower than in the control group. At that time, the average WBC decreased transiently by 56.2% (P < 0.01) but returned to normal values during the second week. Platelet counts were normal, no diarrhea was observed, and none of the rats died (Fig. 1). Administration of analogue AN-238 at the dose of 300 nmol/kg decreased tumor volume by 80.9% (P < 0.01), tumor weight by 82.0% (P < 0.01), and tumor burden by 81.4% (P < 0.01) 4 weeks after the injection (Fig. 2 and Table 1). Single injection of carrier SST analogue RC-121 at 300 nmol/kg did not influence the body weight or tumor growth, and WBC and platelet counts in that group did not differ from the controls. Administration of unconjugated mixture of RC-121 and AN-201 at doses of 300 nmol/kg killed all rats in 4 days.

**Receptor Assay and RT-PCR.** Results of the ligand competition assays on SSTRs are shown in Table 2. In the control group, a single class of specific, high-affinity, and low-capacity binding sites for radiolabeled RC-160 was found, with a mean dissociation constant (Kd) of 9.0 nm and a mean maximal binding capacity (Bmax) of 299.9 fmol/mg membrane protein. In the groups treated with 115 or 300 nmol/kg AN-238, the affinity and capacity of binding sites for RC-160 were not affected, and Kd's and Bmax's were not significantly different from those of controls (Table 2). The IC50 of analogue AN-238 was
healthy tissues. Such compounds could be delivered more selectively to a molecule that can bind to the receptors and a highly active cytotoxic radical. Targeted chemotherapy might improve the responses. The findings of these drugs is strongly limited by their systemic toxicity (6, 7).

Experimental conditions (data not shown).

Using RT-PCR analysis, we detected mRNA for SSTR2 in Dunning R-3327-AT-1 prostate cancer (Fig. 3). Products of the expected sizes of 277 bp for SSTR2 and 261 bp for SSTR5 were found in the control group, as well as AN-238 (300 nmol/kg) Initial 73.3 ± 17.8, AN-238 (115 nmol/kg) 73.4 ± 17.8, AN-238 (300 nmol/kg) 58.9 ± 16.1, and AN-238 (115 nmol/kg) 58.9 ± 16.1. PCR products were subjected to electrophoresis on 8% polyacrylamide gels and stained with silver. The PCR products shown are from one representative tumor from each group. PCR products were of the expected sizes of 277 bp for SSTR2 and 261 bp for SSTR5 were found in rat brain using the same primers and experimental conditions (data not shown).

**DISCUSSION**

During the past several decades, many chemotherapeutic agents have been evaluated in patients with advanced prostatic carcinoma who have become unresponsive to hormonal therapy. However, the overall results have been unsatisfactory because prostate cancer appears to be resistant to most chemotherapeutic agents and because use of these drugs is strongly limited by their systemic toxicity (6, 7). Targeted chemotherapy might improve the responses. The findings that tumor cells can express various membrane receptors makes it possible to design targeted anticancer agents consisting of a carrier molecule that can bind to the receptors and a highly active cytotoxic radical. Such compounds could be delivered more selectively to cancer cells, causing stronger tumor growth inhibition while sparing healthy tissues.

Receptors for SST and mRNAs for SSTR subtypes have been extensively investigated on diverse tumors. These studies revealed that a significant proportion of human tumors can express the octapeptide SST analogue-preferring subtypes SSTR2 and SSTR5 (17, 31). It has also been demonstrated that SST and its octapeptide analogues, such as octreotide or RC-160, can inhibit the growth of some of these tumors (3, 15, 16, 23). Prostatic carcinoma is also known to express SSTRs (9–14), but conflicting data exist about the expression of SSTR2 and SSTR5 subtypes. Although SSTR2 was not found in primary prostate cancer specimens using in situ hybridization, the octreotide-preferring SSTRs were revealed on metastases of hormone-refractory prostatic adenocarcinoma (18, 19). In addition, cultured epithelial cells from prostate cancer specimens were demonstrated to express SSTR5 (9). High-affinity binding of the octapeptides RC-160 and RC-121 was also described on surgically removed human prostate cancer specimens (14).

Recently, we synthesized a powerful cytotoxic SST analogue, AN-238 (31), by linking 2-pyrrolinodoxorubicin, a derivative of doxorubicin that is 500-1000 times more potent in vitro (33), to octapeptide SST analogue RC-121 through an ester bond. In this study, we investigated the antiproliferative effects and toxicity of AN-238, as compared with its components, in Copenhagen rats bearing Dunning R-3327-AT-1 rat prostate cancer. This rapidly growing, androgen-insensitive anaplastic tumor is considered to be representative of the advanced human prostatic carcinoma (41). Inhibitory effects as well as high-affinity binding of RC-160 have been previously shown in this model (24).

Our results demonstrate that AN-238 can significantly inhibit growth of Dunning R-3327-AT-1 tumors in vivo after a single i.v. injection. Analogue AN-238 is much less toxic than its cytotoxic radical AN-201 and more potent in inhibiting tumor growth than AN-201 or the carrier peptide. In the first experiment, the highly potent derivative of doxorubicin, AN-201, had only a weak inhibitory effect on Dunning tumors at the dose of 110 nmol/kg, 4 weeks after a single i.v. injection, and produced no deaths among animals. However, in the comprehensive third experiment, 90% of rats injected with 115 nmol/kg of AN-201 died in 12 days. This suggests that the highest

![Fig. 3. Expression of SSTR2 subtype and β-actin (internal control) in Dunning AT-1 tumors in rats after a single i.v. injection of vehicle (control, Lane 1), RC-121 at 300 nmol/kg (Lane 2), AN-238 at 115 nmol/kg (Lane 3), or AN-238 at 300 nmol/kg (Lane 4), as revealed by RT-PCR. The PCR products shown are from one representative tumor from each group. PCR products were subjected to electrophoresis on 8% polyacrylamide gels and stained with silver. The PCR products were of the expected sizes of 277 bp for SSTR2 and 542 bp for β-actin.](image-url)
nontoxic dose for AN-201 in Copenhagen rats bearing Dunning tumors may be ~110 nmol/kg. Similar variations in toxicity were also found with the methoxymorpholinyl derivative of doxorubicin (FCE 23762), which highly resembles AN-201, in preclinical trials in nude mice (42). Our finding that a single injection of a highly potent cytotoxic agent like AN-201 at sublethal dose had little, if any, effect on the growth of the Dunning AT-1 tumor, is in general agreement with the weak responses seen in patients with advanced stage prostate cancer after systemic administration of many different chemotherapeutic agents, including doxorubicin (6, 7). However, the targeting of cytotoxic radical AN-201 greatly improved the responses of Dunning AT-1 tumors. Thus, cytotoxic SSTR analogue AN-238 at 115 nmol/kg significantly reduced the tumor weight by 42.8% (P < 0.01), indicating that a localized delivery of the cytotoxic radical AN-201 to the tumor tissue can be achieved when it is linked to RC-121. This more selective delivery could be attributed to a targeting action of the SSTR carrier. This view is in accordance with biodistribution studies demonstrating a fast and significant accumulation of an 99mTc-labeled SSTR analogue designed for targeted radiotherapy in pancreatic tumor expressing SSTR (43). AN-238 at the dose of 300 nmol/kg caused a significantly greater reduction in tumor weight than when given at 115 nmol/kg (82.0% versus 42.8%, P < 0.01). Such an effect could be explained by an increased accumulation of the cytotoxic radical in the tumor when a higher dose is given. It is noteworthy that a single injection of AN-238 at a dose of 300 nmol/kg, which is about three times higher than a lethal dose of AN-201, caused no mortality or toxicity. Although it is well known that SSTRs are widely present in various normal tissues, such as the gastrointestinal tract or the pituitary gland (17), it could be speculated that the treatment with cytotoxic SSTR analogue AN-238 does not affect the ability of the undifferentiated or the resting cells in the gastrointestinal tract to replace the damaged cells by mitotic divisions. Preliminary clinical studies have shown no or only low-grade toxicity to the pituitary, the kidney, and the bone marrow after administration of radiolabeled SSTR analogues to cancer patients, whereas a significant reduction in tumor progression was achieved (44, 45).

A single injection of SSTR analogue RC-121 at the dose of 300 nmol/kg had no effect on the tumor growth. This finding is in accordance with the fact that SSTR analogues have to be administered daily in high doses for many weeks or by minipumps to show antitumor activity. Recently, depot formulations of several SSTR analogues have been introduced in clinical trials that can produce high circulating levels of the SSTR analogue for prolonged periods (16).

The radiolabeled ligand competition assay confirmed the presence of specific octapeptide-binding receptors on Dunning AT-1 tumor membranes. Receptor assay also demonstrated a high binding affinity of cytotoxic SSTR analogue AN-238 to these tumors, proving that the binding property of carrier SSTR analogue RC-121 is fully preserved in conjugate AN-238. Because there were no reductions in the concentration and the binding affinity of SSTRs in tumors after administration of AN-238, it is likely that a repetitive treatment with the conjugate could be used to better inhibit tumor growth. Studies with multiple dose regimens are planned for the future. Using RT-PCR analyses, we identified the SSTR2 subtype in the Dunning AT-1 tumors. This subtype is known to bind octapeptide analogues with high affinity (17). On the basis of binding of AN-238 to Dunning AT-1 tumor membranes and the expression of SSTR2, we could conclude that the superior effect of AN-238, as compared to AN-201, and the accompanying lack of toxicity might result from more specific delivery of the cytotoxic conjugate AN-238 to SSTR2 on tumor cells. To confirm this proposed mechanism of action and before any clinical trials, biodistribution studies must be performed with AN-238 and AN-201. Further studies on androgen-independent DU-145 and PC-3 human prostate cancers, as well as on human prostate cancer specimens, are in progress to investigate whether SSTR expression on human prostate cancers is comparable to that found on Dunning AT-1 model.

Our findings suggest that targeted cytotoxic SSTR analogues could be more efficacious and less toxic than presently used systemic chemotherapeutic agents for the therapy of patients with advanced metastatic prostate cancer who no longer respond to androgen deprivation.

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Targeted Cytotoxic Analogue of Somatostatin AN-238 Inhibits Growth of Androgen-independent Dunning R-3327-AT-1 Prostate Cancer in Rats at Nontoxic Doses

Miklos Koppan, Attila Nagy, Andrew V. Schally, et al.

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