Continuous Administration of the Somatostatin Structural Derivative /TT-232/ by Subcutaneously Implanted Osmotic Pump Improves the Efficacy and Potency of Antitumor Therapy in Different Mouse and Human Tumor Models

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Abstract. Background: The somatostatin structural derivative, TT-232, has a special 5-residue ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH₂) and very different characteristics from the known growth hormone (GH) active somatostatin analogs. This somatostatin structural derivative has no GH release inhibitory or antisecretory activity and does not bind to rat pituitary or the cortex, where all the known somatostatin receptor subtypes are expressed. TT-232 had previously been shown to inhibit the proliferation of a large number of cancer cell lines in vitro and reduce the size of different tumors in animal models in vivo. Materials and Methods: The therapeutic efficacy of TT-232 was evaluated in different long-term administration routes: the traditional injection (i.p. or s.c.) versus infusion treatment via s.c.- or i.v.-inserted Alzet osmotic minipump, and on different types of transplantable rodent (S-180 sarcoma, P-388sc lymphoid leukemia, Colon-26 adenocarcinoma, MXT breast carcinoma, B-16 melanoma) and human tumor models (HT-18 lymphoid melanoma, T-47/D breast carcinoma, A-431 epidermoid carcinoma). On the basis of our previous experiments the optimum injected dose of TT-232 was found to be 15 μg/kg twice a day. This dose is equivalent to 0.6 μg/day by infusion therapy. Results: In our experiments, the best results were achieved when TT-232 was applied as an infused treatment. In the S-180 sarcoma and P-388sc lymphoid leukemia rodent tumor models the infusion treatment with TT-232 resulted in 61%-100% tumor growth inhibition and in 20%-60% of the mice being long-term and tumor-free survivors. In the aggressive Colon-26 adenocarcinoma and MXT breast carcinoma models, the infusion treatment resulted in 52%-75% tumor growth inhibition. In the B-16 melanoma model, the infusion treatments resulted in 47%-63% growth inhibition. The tumor growth inhibitory effect of infusion treatment with TT-232 on HT-18 human lymphoid melanoma tumor proved to be significant, resulting in 69%-79% decreases in tumor volume. In the T-47/D human breast carcinoma, the infusion treatment resulted in 48%-53% tumor growth inhibition. The tumor growth inhibitory effect of infusion treatment on A-431 human epidermoid carcinoma tumor resulted in 70%-74% decreases in tumor volume. Conclusion: The antitumor efficacy of TT-232 was seen in almost all the tumors investigated. In our study, the route of infusion was shown to increase drug efficacy relative to conventional delivery methods (injection). The results obtained from this study suggest that TT-232 is a promising new antitumor agent in cancer chemotherapy and a good candidate for delivery by continuous (infusion) therapy.

Somatostatin is a naturally occurring cyclic tetradecapeptide hormone produced mostly by the hypothalamus and characterized as a regulatory-inhibitory peptide with exocrine, endocrine, paracrine and autocrine activity. It inhibits or regulates several cell functions, including the inhibition of secretory and proliferative processes, and is also considered as an important endogenous antitumor agent (1-2). Somatostatin and its analogs have attracted intense attention in drug development and reached clinical application in the 1990s. Analogs of somatostatin have already been used in clinical practice but many follow-up molecules are still in the exploratory phase and under clinical development (3-5). A new potent tumor-selective somatostatin structural derivative, TT-232, was developed and reported by us (6-9).
TT-232 contains a five-residue ring (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH₂) and shows unique conformational characteristics compared to traditional somatostatin analogs. Historically, development of TT-232 was started as a signal-transduction inhibitor drug candidate targeting oncological applications in 1992, while increasing evidence as to the molecular pharmacology of its action, along with extensive preclinical efficacy studies, have also brought its anti-inflammatory indication into the front line of present development efforts. TT-232 inhibited the tyrosine kinase activity of tumor cells and this inhibition correlated well with the inhibition of cell proliferation. The antitumor activity of TT-232 has been associated with induction of programmed cell death (apoptosis) in tumor cells, resulting in highly selective elimination of neoplastic tissue. TT-232 induced apoptosis in a time- and dose-dependent manner and inhibited mitosis of the cell population, which paralleled apoptosis, by both biochemical and morphological parameters (10-13).

The molecular mechanism of these biological activities has been linked to both short-term activation of intracellular tyrosine phosphatases and long-term inhibition of tyrosine kinase. The role of tyrosine kinase inhibition in the induction of apoptosis has been well demonstrated, while our recent studies proved that an endothelial growth factor receptor (EGFR)-selective tyrosine kinase inhibitor induced non-apoptotic programmed cell death (14-15). TT-232 was reported to bind to somatostatin receptors (SSTR-1 and SSTR-4) with high affinity. Short-term (30 min) exposure of cells to TT-232 activated SSTR receptors (primarily SSTR-1), which led to irreversible cell cycle arrest in the G1/S-phase followed by secondary induction of apoptosis (16-18). In contrast, continuous incubation with TT-232 led to direct induction of active cell death, independently of SSTR-mediated signaling (19-20). The antitumor effect of TT-232 is mediated through the SSTR-1 receptor and by the tumor-specific isoform of pyruvate kinase. Similarly to natural somatostatin (21), TT-232 induces early transient activation of the ERK/MAPK pathway, which leads to the induction of the cyclin-dependent kinase inhibitor p21<sup>cip1/Waf1</sup>, causing irreversible cell cycle arrest in the G1/S phase (22).

TT-232 has passed phase I clinical trials without toxicity or significant side-effects, and phase II studies are in progress for oncological and anti-inflammatory indications. The aim of our study was to investigate the antitumor efficacy of TT-232 in different long-term administration routes: intermittent (injection) versus continuous (infusion) treatment via s.c.- and i.v.- inserted Alzet osmotic minipump in different rodent and human tumor models. A great number of in vivo experiments using various tumor types were conducted to define the in vivo tumor growth inhibitory properties of TT-232. Extensive studies also targeted the relationship between the efficacy and the route of administration/duration of treatment.

Materials and Methods

Compound. TT-232, a somatostatin structural derivative, was dissolved in buffer solution (pH 4.1) containing 0.1 M acetic acid, 0.1 M sodium acetate and 3% mannitol diluted with distilled water. The solution of TT-232 proved to be stable at 37°C for 3 weeks.

Animals. All animal work was performed in a specified pathogen-free (SPF) breeding house of the animal facility of the Department of Experimental Pharmacology, National Institute of Oncology (Budapest, Hungary). The animals used in these studies were cared for according to the “Guiding Principles for the Care and Use of Animals” (23-24) based upon the Helsinki declaration and which were approved by the local Ethical Committee. The animals were fed with a sterilized standard diet (Biofarm, Budapest, Hungary) and had free access to tap water ad libitum. They were kept in macrolon cages at 23-25°C (40%-50% humidity), with a lighting regimen of 12/12 hours light/dark. In our experiments 5-10 mice per group were utilized.

Treatment conditions. Injection: intraperitoneal (i.p.) and subcutaneous (s.c.) treatment. Infusion: s.c.-with the s.c. inserted Alzet osmotic minipump and i.v.-via a catheter (Model PE-10). Alzet minipump can deliver directly into the external jugular vein.

Route of administration and treatment schedule of TT-232. The antitumor effect of the somatostatin structural derivative TT-232 was studied using different routes of administration and treatment schedules in rodent and human tumor models. Treatment with TT-232 started 1 day after tumor transplantation or after development of the tumor (on 7th day). On the basis of our previous experiments (25-26), the optimum dose of injected TT-232 was determined to be 15 μg/kg twice a day. This dose is equivalent to 0.6 μg/day by infusion therapy. In all cases, vehicle solution was used as control. Ratio of the volume/body weight: 0.1 ml/10 g. In the experiments, we studied the therapeutic effect of the TT-232 with i.p. and s.c. injection over 14 days (1xqdx2wxd) and for 7- and 14-days s.c. and i.v. infusion treatment employing an Alzet osmotic minipump (Model 2001 or 2002). The therapeutic effect of TT-232 in two human tumor models (T-47/D breast carcinoma and A-431 epidermoid carcinoma) with i.p. and s.c. injection over 30 days (30xqd) and 14- and 28-day s.c. infusion treatment with the application of Alzet osmotic minipumps (Model 2002) were investigated.

Osmotic minipump. The Alzet osmotic minipump (Model 2001 or 2002) was obtained from the Alzet Corporation (Palo Alto, CA, USA). The Alzet osmotic minipumps with a reservoir of 200 μl capacity with a mean pumping rate of 1.0 μl/h or 0.5 μl/h were used at 1 week or 2 weeks' duration. The Alzet minipump model 2001 delivers 200 μl over a 1-week period, whereas the Alzet model 2002 delivers 200 μl over 2-week period. In the case of 28 days of TT-232 treatment, two Alzet osmotic minipumps were utilized successively. The administration of TT-232 with the osmotic minipump was carried out as instructed by the manufacturer (27-28). The animals were anesthetized by sodium-pentobarbital (Nembutal®, Abbot Lab., Ceva, Paris, France) at a dose of 50 mg/kg, i.p. The usual site for subcutaneous implantation of Alzet minipump in mice is on the back, slightly posterior to the scapulae. Continuous infusion via Alzet osmotic minipump is feasible only when the administered
drugs is stable throughout the delivery period. The stability of TT-232, both in solid (lyophilized) form and in aqueous solution, was investigated during storage at different temperatures. Samples were stored for various time-periods and analyzed for TT-232 content as well for degradation products using HPLC methods (29).

**Evaluation.** The body weight of animals was measured and the tumor dimensions were measured with a microcaliper on every second or third day. The tumor volume was calculated with the following formula: \( V = (\pi d^2)/4 \times b \) (V: tumor volume, d: longest diameter, b: diameter perpendicular to L). Survival times related to that of the controls were recorded. Tumor volume measurements were continued until the first death in the control group. Mean values and standard deviations (S.D.) were calculated. Experimental data were subjected to computerized statistical analysis of variance with the Student-Newman-Keuls test; statistical significance was accepted at \( p<0.05 \) levels (30).

**Results**

**Influence of the different administration routes on the therapeutic effect of TT-232 in different rodent tumor models.**

Table I shows the significant inhibitory effect of TT-232 in different rodent tumor models. When TT-232 was given a dose of 15 \( \mu \)g/kg, by i.p. or s.c. injection, twice a day for 2 weeks the tumor growth inhibitory effect was 31% and 32%, respectively. By means of i.p. and s.c. injection treatment with TT-232, we achieved a cure rate of 30%. The TT-232 administered via the s.c. minipump for 7 days evoked a significant (77%) tumor inhibitory effect. The continuous two-week infusion of the TT-232 using s.c. and i.v. inserted minipumps resulted in 100%-80% growth-inhibitory effect and tumor-free survival in 60% and 40% of the treated animals, respectively. When TT-232 treatment started after the development of tumor, the tumor growth-inhibitory effect of injection treatment was 23% (i.p.) and 24% (s.c.), respectively. The s.c. infusion treatment for 14 days period resulted in 61% tumor inhibitory effect. The strongest tumor growth-inhibitory effect (96%) was achieved when the minipump was inserted i.v. for 2 weeks. The s.c. and i.v. infusions applied for a longer duration (2 weeks) produced 40% long-term and tumor-free survival of TT-232 treated mice. When TT-232 was applied for P-388sc lymphoid leukemia tumor for 2 weeks, the tumor growth-inhibitory effect was 59% (i.p. injection) and 58% (s.c. injection). When TT-232 was administered via s.c. minipump for 14 days TT-232 induced a significant (67%) tumor growth-inhibitory effect. Significant (76%) growth-inhibitory effect was obtained by the s.c. infusion for 28 days. A total (100%) tumor growth-inhibitory effect was achieved by the i.v. infusion treatment for 28 days. In the following 28 days of s.c. infusion treatment with TT-232, 20% of the animals became free of the tumor. After the 28 days of i.v. infusion of the TT-232 40% of the animals were tumor free. When TT-232 treatment started after the development of tumor, the long-term TT-232 treatment given as i.p. or s.c. injection influenced the growth of P-388sc tumor to different extents (61% and 25%). Two 28-day infusion (s.c. and i.v.) treatments resulted in 81% and 82% tumor inhibition and produced tumor-free, long-term survivors in 20% of all mice. When we applied injection treatment of TT-232 for Colon-26 adenocarcinoma tumor for 14 days, a significant (i.p. 48%, s.c. 44%) tumor growth-inhibitory effect was achieved. Infusion treatment of TT-232 for 14 and 28 days applied s.c. and i.v. drastically inhibited tumor growth (52% and 75%). When treatment started after the development of tumor, TT-232 injected i.p. or s.c. influenced moderately (25%) the growth of colon-26 tumor. The best result was achieved by the i.v. infusion for 28 days (60% tumor growth-inhibitory effect). When we applied TT-232 for MXT carcinoma tumor in s.c. injection for 14 days, a significant (39%) tumor growth-inhibitory effect was achieved. The s.c. infusion of TT-232 for 14 and 28 days drastically inhibited the tumor growth of tumor-bearing mice (65% and 71%). After the development of tumor, the injection (i.p/s.c.) treatment had a moderate (26%) tumor growth-inhibitory effect. The s.c. and i.v. infusion treatment for 28 days, respectively, resulted in 62% and 70% tumor inhibitory effects. When we applied TT-232 for B-16 melanoma tumor at a dose of 15 \( \mu \)g/kg, i.p. or s.c. injections for 14 days, the tumor growth-inhibitory effect was 35% and 39%, respectively. TT-232 administered via the s.c. minipump for 7 days evoked a significant (47%) tumor growth inhibition. When TT-232 was administrated via s.c. infusion for 14 days, TT-232 induced a significant (57%) tumor growth-inhibitory effect. A significant (63%) growth-inhibitory effect was obtained by the s.c. infusion for 28 days.

**Influence of the different administration routes on the therapeutic effect of TT-232 in different human tumor models.**

Table I shows the tumor growth-inhibitory effect of TT-232 in different human tumor models. The tumor inhibitory effect of TT-232, via injection and infusion treatment in the HT-18 human melanoma tumor model was investigated. When TT-232 was applied via injection treatment for 14 days, the melanoma tumor growth-inhibitory effect was 41% (i.p.) and 63% (s.c.) respectively. The s.c. infusion treatment of TT-232 for 7 and 14 days resulted in a 69% and 73% tumor inhibitory effect, respectively. The strongest tumor growth-inhibitory effect (79%) was achieved when the Alzet osmotic minipump was inserted s.c. for 28 days. When TT-232 was applied for T-47/D human breast carcinoma tumor at a dose of 15 \( \mu \)g/kg by s.c. and i.p. injection for 30 days (30xq3d) a moderate (23% and 26%) tumor growth-inhibitory effect was observed. TT-232 administrated via the s.c. Alzet minipump for 14 days evoked a significant (48%) tumor growth-inhibitory effect. In the case of s.c.-implanted Alzet minipumps used for 28 days, a 53% tumor growth-inhibitory effect was achieved. When we applied TT-232 treatments for
A-431 human epidermoid carcinoma tumor at a dose of 15 μg/kg for 30 days (30xq), the tumor growth-inhibitory effect was 35% (s.c. injection) and 43% (i.p. injection), respectively. The s.c. infusion treatment for 14 and 28 days, respectively, resulted in 70% and 74% tumor growth inhibitory effects.

**Discussion**

The somatostatin structural derivative TT-232 represents a novel approach from traditional chemotherapeutic regimens in the treatment of certain types of cancer. The promising advantages of TT-232 treatment include a wide range of inhibitory and antiproliferative actions with few side-effects. In the present study, the therapeutic efficacy of the somatostatin structural derivative TT-232 was evaluated in various long-term administration routes, namely the traditional intermittent (injection) versus continuous (infusion) treatment via a s.c.-inserted Alzet osmotic minipump, on different rodent and human tumor models. The results of our experiments demonstrated that much better results were obtained with the application of low doses of TT-232 in continuous (infusion) treatment than the application by intermittent (injection).
treatment. When the total amounts of TT-232 applied by the different administration routes (injection and infusion) are compared, it is evident that the infusion treatment route could significantly increase the specific activity of TT-232. The comparative experiments confirmed that continuous treatments and long-term administration were associated with the best treatment responses in both of the in vivo models studied. Our results and the data of other authors (31-35) demonstrate that the therapeutic doses given by infusion achieved significantly greater reductions in tumor size than identical doses given by either of the injection schedules. The frequent and long-lasting repetition of TT-232 injection enhanced its therapeutic efficacy; however, serial injection causes significant stress to animals and adequate precautions are required. To this end, an Alzet osmotic minipump inserted s.c. and i.v. was used. Infusion through the inserted Alzet osmotic minipump maintained a constant drug level and resulted in a well-defined, consistent pattern of drug exposure throughout the period of drug administration, which suggests the potential benefits of TT-232 in clinical practice.

By extrapolating these results for human clinical application, continuous infusion therapy can be regarded as most promising in terms of ease of application and predicted efficacy. The development of the optimum treatment schedule and the significant sensitivity to TT-232 in the tested rodent and human tumors represent promising data for human clinical trial. The results obtained from this study suggest that TT-232 is a good candidate for delivery by continuous (infusion) therapy.

References


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