Antitumor Activity of the Somatostatin Structural Derivative (TT-232), Against Mouse and Human Melanoma 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. TT-232 inhibited tyrosine kinase activity of tumor cell lines and this inhibition correlated well with the inhibition of cell proliferation of a large number of cancer cell lines in vitro and reduces the size of different tumors in animal models in vivo. The antitumor efficacy of TT-232 has been found to be associated with the induction of apoptosis in tumor cells, resulting in highly selective elimination of tumor tissue. TT-232 was found to be devoid of GH release inhibitory activity but to possess strong antitumor effects. It binds with a high affinity to SSTR1 and SSTR4. This compound was also found to inhibit inflammation in a number of experimental models. Materials and Methods: The study compared the antitumor effect of TT-232 in various long-term administration routes: an intermittent (injection) versus continuous (infusion) treatment via subcutaneously inserted 2002 type Alzet osmotic minipumps in two different tumor models (B-16 rodent melanoma and HT-18 human lymphoid melanoma). Treatment with TT-232 started after disease development. The antitumor efficacy of TT-232 was evaluated on the basis of tumor growth inhibition and survival time. Results: In the case of B-16 rodent melanoma, the TT-232 treatments resulted in 35%-39% (injection) and 47%-63% (infusion) tumor growth inhibition, and the infusion treatment an approximately 61% increase in survival time. The tumor growth inhibitory effect of TT-232 on HT-18 lymphoid melanoma proved to be significant, resulting in 41%-63% (injection) and 69%-79% (infusion) decreases in tumor volume and in a 25%-30% increase in survival time (infusion treatments). Conclusion: The results indicate that TT-232 could be a potentially useful therapeutic agent if these data are translated into clinical practice.

Somatostatin and its structural analogs have been shown to have a therapeutic potential for various neuroendocrine neoplasms (1), as well as in the clinical relief of esophageal bleeding in portal hypertension (2). All these effects have been linked to the presence of cell-surface receptors of somatostatin (SSTR1-4) and extensive research was directed toward development of selective analogs with an even longer half-life for more straightforward clinical application. TT-232 is a structural derivative of somatostatin with a five-ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH2) exhibiting a strong and selective growth inhibitory effect. The antitumor effect is mediated through the SSTR1 receptor and by the tumor-specific isoform of pyruvate kinase. Its mechanism of action is in line with a new era of molecular medicine called signal transduction therapy, where "false" intracellular or intercellular communication is inhibited or corrected without interfering with basic cell functions and machinery. 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. TT-232 is the first somatostatin structural derivative with a selective antitumor potential without antisecretory activity synthesized and patented by our group. TT-232 has been shown to inhibit proliferation and induce apoptosis both in vitro and in vivo in various types of tumor cells but also inactivated lymphocytes. 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 (3). Short-term (30-min) exposure of cells to TT-232 activated SSTR receptors (primarily SSTR-1), which led to irreversible cell cycle arrest in G1/S-phase followed by secondary induction of apoptosis (4-6). In contrast, continuous incubation with TT-232 led to
direct induction of active cell death, independently from SSTR-mediated signaling (7). The mechanistic studies of the TT-232 have been already described in our previous publications (8-17). Here, the therapeutic efficacy of TT-232 was studied on the basis of survival and tumor growth inhibition, using two melanoma tumor models and applying different administration routes and treatment schedules. Till now tumor efficacy of TT-232 was not investigated on a melanoma tumor model. The present study was undertaken to define the optimal route for TT-232 administration and to evaluate the antitumor effect of such treatment in two different melanoma tumor models: B-16 rodent melanoma and HT-18 human lymphoid melanoma tumor. During our experiments, we evaluated the antitumor efficacy of TT-232 in various long-term administration routes: traditional injection versus infusion treatment via an inserted Alzet minipump (Model 2002). Continuous (infusion) administration of TT-232 significantly inhibited the growth of the tumor when compared to intraperitoneal (i.p.) and subcutaneous (s.c.) intermittent (injection) treatments.

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 treated according to the “Guiding Principles for the Care and Use of Animals” (18) 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, 10 mice per group were utilised.

Tumor cells. B-16 rodent melanoma cells and HT-18 human lymphoid melanoma cells (obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA) were used.

Transplantation of the tumors. B-16 rodent melanoma cells 5x10^6 per mouse and an optimal fragment (1-5x10^5 mg) of HT-18 human lymphoid melanoma tumor/mouse were transplanted subcutaneously (s.c.) into the intrascapular region of the mouse. Treatment with TT-232 started after development of the tumor. In all cases vehicle solution was used as control. Ratio of the volume/body weight: 0.1 ml/10 g (s.c.).

Osmotic minipump. The Alzet osmotic minipump (Model 2002, delivering 1.0 ml/h for 14 days) was obtained from the Alza Corporation (Palo Alto, Ca, USA). The administration of TT-232 with the osmotic minipumps was carried out as instructed by the manufacturer (19-20). The animals were anesthetized with sodium-pentobarbital (Nembutal®, Abbot Lab., Ceva, Paris, France) at a dose of 50 mg/kg, i.p. In the case of 28 days of TT-232 treatment, two Alzet osmotic minipumps were utilized successively. Continuous infusion via Alzet minipumps is feasible only when the administered drug is stable throughout the delivery period. The stability of TT-232, both in a solid (lyophilized) form and in aqueous solution, was investigated during storage at different temperatures. Samples, stored for various time-periods, were analyzed for TT-232 content as well for degradation products using HPLC methods (21).

Administration route and treatment schedule. The i.p. and s.c. injections were applied twice a day for 14 days (14xq12hx2d). On the basis of our previous experiments, it was determined that the optimum dose of TT-232 was 15 µg/kg twice a day for injection treatment. The injection dose (15 µg/kg twice a day) equals a 0.6 µg/day infusion treatment with an Alzet osmotic minipump (Model 2002).

Evaluation. The body weight of animals was weighed and the tumor volumes were measured with a microlipper on every second or third day. The tumor volume was calculated with the following formula: V=(π/6)xLxD² (V: tumor volume, L: longest diameter, D: 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 (X) and standard deviations (S.D.) were calculated. Experimental data were subjected to computerised statistical analysis of variance with the Student-Newman-Keuls test; statistical significance was accepted at p<0.05 levels.

Results

The antitumor efficacy of TT-232 on B-16 rodent melanoma tumor. The antitumor activities of TT-232 through intermittent (injection) and continuous (infusion) treatments were compared. When TT-232 was given at a dose of 15 µg/kg, by i.p. or s.c. injection, twice a day 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 compared to the control. When TT-232 was administrated via s.c. minipump for 14 days TT-232 induced a significant (57%) tumor growth-inhibitory effect. Significant (63%) growth-inhibitory effect was obtained by the s.c. infusion for 28 days. The antitumor efficacy of TT-232 treatment on survival time of B-16 tumor-bearing mice is presented in Table I. The s.c. infusion therapy for 7 days resulted in a moderately increased (by 46%) survival time of the tumor-bearing animals. The infusion with the s.c. minipump for 14 or 28 days significantly increased (by 58% and 76%, respectively) the long-term survival of tumor-bearing mice.

The antitumor effect of TT-232 on HT-18 human lymphoid melanoma tumor. The tumor inhibitory efficacy of TT-232, via injection and infusion treatment in the HT-18 human...
TT-232, a somatostatin structural derivative with a five-ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH₂), was developed by this laboratory and published in an earlier work (22, 23). Regarding the mode of action, TT-232 activates cell cycle inhibitors via SSTR receptors, inhibits tyrosine kinases through interfering with the proliferative signaling cascades, and interacts with an intracellular receptor and an enzyme involved in glycolysis causing translocation of this enzyme to the nucleus, thus inducing apoptosis (24, 26). The aim of our experiments was to evaluate the therapeutic efficacy of TT-232, in various long-term administration routes; traditional injection versus infusion treatment via an s.c.-inserted minipump on two melanoma tumor models (B-16 rodent melanoma and HT-18 lymphoid human melanoma). A long-term infusion of TT-232 with the Alzet minipumps (Model 2002) was used in order to maintain a low dose of the peptide in the circulation for a longer time-period. Our results and the data of other authors (27-36) demonstrated that the therapeutic doses given by infusion achieved significantly greater reductions in tumor size than the identical doses given by either of the intermittent (injection) schedules. In the studies of the tumor growth-inhibitory effect of TT-232, the best results were achieved when TT-232 was applied by continuous (infusion) treatment. In the case of B-16 rodent melanoma tumor model, the infusion treatments drastically inhibited the tumor growth and increased survival time about 60%. In the HT-18 human lymphoid melanoma tumor-bearing mice a significant growth-inhibitory effect was obtained by the s.c. infusion treatment and increased survival time with about 25%. The comparative experiments confirmed that continuous treatments and long-term administration were associated with the best treatment responses in both the in vivo models studied. The frequent and long-lasting repetition of TT-232 injection enhanced its therapeutic efficacy, however, serial injections cause significant stress to animals and adequate precautions are required. To this end, an Alzet osmotic minipump inserted s.c. was used. Infusion through the inserted Alzet osmotic minipumps maintained a constant drug level and resulted in a well-defined, consistent pattern

### Table I. Effect of the different administration routes and treatment schedules on the therapeutic efficacy of TT-232 in two melanoma tumor models.

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Treatment</th>
<th>Dose</th>
<th>Treatment Schedule</th>
<th>Route</th>
<th>Mean survival</th>
<th>Tumor inhibitory effect of TT-232</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>M±SD</td>
<td></td>
<td>T/C (%)</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cm³</td>
<td>% of control</td>
<td>% P</td>
<td></td>
</tr>
<tr>
<td>B-16</td>
<td>Injection</td>
<td>15 Ìg/kg</td>
<td>10x12hx2d</td>
<td>i.p.</td>
<td>19.8±2.5</td>
<td>111.2 n.s.</td>
</tr>
<tr>
<td>rodent</td>
<td></td>
<td>15 Ìg/kg</td>
<td>10x12hx2d</td>
<td>s.c.</td>
<td>21.1±2.3</td>
<td>118.5 n.s.</td>
</tr>
<tr>
<td>melanoma</td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>7 days</td>
<td>s.c.</td>
<td>25.9±4.7</td>
<td>145.5 &lt;0.025</td>
</tr>
<tr>
<td></td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>14 days</td>
<td>s.c.</td>
<td>28.1±3.9</td>
<td>157.8 &lt;0.025</td>
</tr>
<tr>
<td></td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>28 days</td>
<td>s.c.</td>
<td>31.3±3.5</td>
<td>175.8 &lt;0.025</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
<td></td>
<td>-</td>
<td>17.8±2.2</td>
<td>100.0 -</td>
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<tr>
<td>HT-18</td>
<td>Injection</td>
<td>15 Ìg/kg</td>
<td>10x12hx2d</td>
<td>i.p.</td>
<td>160.0±2.5</td>
<td>106.6 n.s.</td>
</tr>
<tr>
<td>human</td>
<td></td>
<td>15 Ìg/kg</td>
<td>10x12hx2d</td>
<td>s.c.</td>
<td>172.8±2.9</td>
<td>115.2 n.s.</td>
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<tr>
<td>lymphoid</td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>7 days</td>
<td>s.c.</td>
<td>178.6±5.8</td>
<td>119.0 n.s.</td>
</tr>
<tr>
<td>melanoma</td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>14 days</td>
<td>s.c.</td>
<td>187.0±3.6</td>
<td>124.6 &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Infusion</td>
<td>0.6 Ìg/day</td>
<td>28 days</td>
<td>s.c.</td>
<td>195.0±3.9</td>
<td>130.0 &lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>-</td>
<td></td>
<td>-</td>
<td>150.2±3.8</td>
<td>100.0 -</td>
</tr>
</tbody>
</table>

*Treatments were started after the development of tumor; mp=Alzet osmotic minipump.
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 term of ease of application and predicted efficacy. Development of the optimum treatment schedule and the significant sensitivity of the tested human tumors to TT-232 represent promising data for human clinical trials. The results obtained from this study suggest that TT-232 is a good candidate for delivery by continuous infusion therapy.

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References


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