Growth Inhibitory Effect of the Somatostatin Structural Derivative (TT-232) on Leukemia Models

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Abstract. TT-232 is a structural derivative of the natural signal inhibitory peptide somatostatin, with selective antiproliferative and anti-inflammatory properties. TT-232 activates SSTR receptors (primarily the SSTR-1), which leads to irreversible cell cycle arrest, followed by secondary induction of apoptosis. TT-232 has passed phase I clinical trials without toxicity and significant side-effects. We examined the antiproliferative effect in vitro and the antitumor effect in vivo of TT-232 on leukemia cell lines. During in vivo experiments, we evaluated the therapeutic efficacy of TT-232 in various long-term administration routes; traditional injection versus infusion treatment via an inserted Alzet minipump on P-388 mice and HL-60 human leukemia models. Treatment with TT-232 started after development of the disease. In vitro, TT-232 inhibited the proliferation of P-388 mice lymphoid cells and HL-60 human promyelocytic leukemia cells in the range of 46%-97% with 24-hour treatment and 82%-100% with 48-hour treatment. Cells were treated with 30 μg/ml and 60 μg/ml dose of TT-232. With the same in vivo models, the best results were achieved when TT-232 was applied by infusion treatments. The infusion treatment with TT-232 produced 50%-80% inhibition of growth and resulted in 20%-40% long-term and leukemia-free survivors. TT-232 showed dose-, time- and administration mode-dependent antileukemia activity in vitro and in vivo, both on rodent and human models. Our results suggest that TT-232 is a promising new antileukemia agent.

The present study was designed to examine the efficacy of the novel somatostatin analog (TT-232) in various leukemia models. Somatostatin, a natural tetradecapeptide, inhibits both the growth hormone release and various endocrine secretions (i.e. glucagon, insulin, gastrin). It inhibits or regulates several cell functions also being an important endogenous antitumor agent (1-4). TT-232 is a structural heptapeptide analog of somatostatin, (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH2) but, in contrast to the parent hormone and its "traditional" analogs, this compound has strong and selective growth-inhibitory antitumor potential without the wide-ranging endocrine side-effects. It also has a strong anti-inflammatory and neurogenic inflammation inhibitory activity. The molecule has been shown to have unique conformational characteristics, selective binding properties to the 1st and 4th subtypes of somatostatin receptors (SSTR1 and 4) and to the intracellular receptor: pyruvate kinase M2. Its mechanism of action is in line with the new era of molecular medicine called signal-transduction therapy, where "internal communication" of cells is corrected without interfering with basic cell functions and machinery. 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 in activated 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 kinases (5). Short-term (30 min) exposure of cells to TT-232 activates SSTR receptors (primarily the SSTR-1), which leads to irreversible cell cycle arrest in G1/S followed by secondary induction of apoptosis (6). In contrast, continuous incubation with TT-232 leads to direct induction of active cell death independently from SSTR-mediated signaling (7). The mechanism of action and the signaling cascade of TT-232 in A431 epidermoid carcinoma cells has been fully elucidated (8-11). In our previous in vitro and in vivo experiments, the antitumor efficacy of the novel somatostatin analog was studied on different tumor models (12-17). The antitumor activity of TT-232 has been found to be associated with induction of programmed cell death (apoptosis) in tumor cells, resulting in highly selective elimination of tumor tissue. TT-232 induced apoptosis in a time- and dose-dependent manner and inhibited mitosis of the cell population, that paralleled apoptosis by both biochemical and morphological parameters (18-20). The TT-232-induced inhibition of the
growth-promoting tyrosine kinase signal could be coupled to the induction of the regular cell cycle with an apoptotic end. The role of tyrosine kinase inhibition in the induction of apoptosis has been well demonstrated, while our recent studies proved that an EGFR selective tyrosine kinase inhibitor induced a non-apoptotic programmed cell death. Tyrosine kinase inhibitors and signal transduction therapy opens a new era in the treatment of leukemia, representing the first targeted molecular therapy which is able to target abnormal cells without damaging normal cells, as compared with traditional antineoplastic drugs (21-23).

The objectives of these studies were to investigate the therapeutic efficacy of TT-232 on rodent and human leukemia models in vitro and in vivo. In vitro, we investigated the effect of TT-232 on P-388 mice lymphocytic and HL-60 human promyelocytic leukemia cell lines. Cells were treated with a 30 µg/ml and 60 µg/ml dose of TT-232 for 24 and 48 hours. In vivo, we studied the therapeutic efficacy of the novel somatostatin analog on P-388 mice and HL-60 human tumor-bearing mice applying the injection and infusion treatments of TT-232. The antineoplastic activity of TT-232 was evaluated bearing mice applying the injection and infusion treatments of TT-232. The antineoplastic activity of TT-232 on rodents and human leukemia models in vitro and in vivo. In vitro, we investigated the effect of TT-232 on P-388 mice lymphocytic and HL-60 human promyelocytic leukemia cell lines. Cells were treated with a 30 µg/ml and 60 µg/ml dose of TT-232 for 24 and 48 hours. In vivo, we studied the therapeutic efficacy of the novel somatostatin analog on P-388 mice and HL-60 human tumor-bearing mice applying the injection and infusion treatments of TT-232. The antineoplastic activity of TT-232 was evaluated bearing mice applying the injection and infusion treatments of TT-232. The antineoplastic activity of TT-232 on rodents and human leukemia models in vitro and in vivo.

Table I. In vitro antiproliferative effect of TT-232 on leukemia cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Inhibition of cell proliferation (%)</th>
<th>24 hours</th>
<th>48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-388 rodent lymphocytic leukemia</td>
<td></td>
<td>46±2</td>
<td>82±3</td>
</tr>
<tr>
<td>HL-60 human promyelocytic leukemia</td>
<td></td>
<td>59±3</td>
<td>97±2</td>
</tr>
</tbody>
</table>

The applied doses of TT-232 were 30 and 60 µg/ml. The treatment periods were 24 and 48 hours. Values are the mean±SD.
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Figure 1. In vitro activity of TT-232 on different leukemia cell lines.

Figure 2. Antitumor efficacy of the somatostatin analog (TT-232) on rodent and human leukemia tumor models.

Statistical analysis: experimental data were subjected to computerized statistical analysis of variance with the Student-Newman-Keuls test; statistical significance was accepted at \( p < 0.05 \) level. Experiments were performed with a group of 5-10 mice.
Results

**In vitro studies.** Figure 1 and Table I show the significant antiproliferative effect of the novel somatostatin structural derivative (TT-232) on P-388 mice lymphocytic and HL-60 human promyelocytic leukemia cell lines. When the P-388 cell line was treated with TT-232 for 24 hours at doses of 30 Ìg/ml and 60 Ìg/ml, the number of tumor cells was decreased by 46±2% and 82±3%. In the case of treatment for 48 hours, the inhibition caused by TT-232 was about 59±3% and 97±2%. On the HL-60 cell line, 30 and 60 Ìg/ml doses of TT-232 for 24 hours produced a very significant antiproliferative effect (82±1 and 92±1%). When this line was treated with 30Ìg/ml and 60Ìg/ml doses of TT-232 for 48 hours, a dramatic inhibition of the growth of HL-60 leukemia cells was achieved (99±0% and 100±0%).

**In vivo studies.** The antitumor effect of TT-232 on P-388 rodent lymphocytic leukemia tumor: We investigated the inhibitory effect of TT-232 via injection and infusion treatment on a HL-60 promyelocytic leukemia tumor model. When we applied TT-232 at a dose of 15 mg/kg with s.c. injection for 14 days, a moderate (26%) tumor growth-inhibitory effect was observed. The 3 mg/kg and 0.3 mg/kg, s.c. injection treatment for 14 days resulted in 32% and 44% tumor inhibitory effects. On the basis of tumor growth curves, a significant inhibitory activity of this novel somatostatin analog (TT-232) was observed following long-term infusion using Alzet 2002 tip. osmotic minipumps implanted s.c. The tumor inhibitory activity of TT-232 following infusion treatment for 28 days was 50% (3 Ìg/day) and 60% (12 Ìg/day) with Alzet tip. implanted minipumps (Figure 2B). The effect of TT-232 on survival times is presented in Table II. The s.c. infusion of TT-232 using s.c. implanted Alzet tip. osmotic minipumps for 28 days resulted in tumor-free survival in 40% (3 Ìg/day) and in 20% (12 Ìg/day) of the treated animals, respectively.

**Table II. Influence of the different administration routes and treatment schedules on the therapeutic effect of the somatostatin structural derivative (TT-232) in rodent and human leukemia models.**

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Treatment</th>
<th>Dose</th>
<th>Treatment Schedule</th>
<th>Treatment route</th>
<th>Survival time</th>
<th>T/C %</th>
<th>Survivors/ Total</th>
<th>Survival T/C x 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-388sc rodent lymphocytic leukemia</td>
<td>injection</td>
<td>15 Ìg/kg</td>
<td>12hx2dx14</td>
<td>i.p.</td>
<td>20.8±1.3/10 mice</td>
<td>112</td>
<td>0/10 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>19.8±1.6/10 mice</td>
<td>106</td>
<td>0/10 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>infusion</td>
<td>0.6 Ìg/day</td>
<td>14 days</td>
<td>s.c.</td>
<td>23.0±2.6/10 mice</td>
<td>115</td>
<td>0/10 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>0.6 Ìg/day</td>
<td>28 days</td>
<td>s.c.</td>
<td>33.0±2.1/8 mice</td>
<td>165²</td>
<td>2/10 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>34.8±3.3/8 mice</td>
<td>174²</td>
<td>2/10 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>infusion</td>
<td>0.3 mg/kg</td>
<td>qd x 14</td>
<td>s.c.</td>
<td>61.6±1.7/5 mice</td>
<td>144</td>
<td>0/5 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>injection</td>
<td>3 mg/kg</td>
<td>qd x 14</td>
<td>s.c.</td>
<td>56.4±1.4/5 mice</td>
<td>132</td>
<td>0/5 –</td>
<td></td>
</tr>
<tr>
<td>HL-60 human promyelocytic leukemia</td>
<td>infusion</td>
<td>15 mg/kg</td>
<td>qd x 14</td>
<td>s.c.</td>
<td>53.9±1.6/5 mice</td>
<td>126</td>
<td>0/5 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>42.8±1.2/5 mice</td>
<td>100</td>
<td>0/5 –</td>
<td></td>
</tr>
<tr>
<td></td>
<td>infusion</td>
<td>3 Ìg/day</td>
<td>28 days</td>
<td>s.c.</td>
<td>47.0±3.2/3 mice</td>
<td>105</td>
<td>2/5 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>–</td>
<td>12 Ìg/day</td>
<td>28 days</td>
<td>s.c.</td>
<td>48.0±1.2/4 mice</td>
<td>107</td>
<td>1/5 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>44.7±3.9/5 mice</td>
<td>100</td>
<td>0/5 –</td>
<td></td>
</tr>
</tbody>
</table>

1Tumor-free
2Statistical significance was accepted at p<0.05 level. Experiments were performed with a group of 5-10 mice.
TT-232 has been developed as a signal-transduction inhibitor drug candidate targeting oncological applications, while increasing evidence on the molecular pharmacology of its action, along with extensive preclinical efficacy studies, have demonstrated a strong anti-inflammatory effect and potential therapeutic indication also(26-27). The overall results of the numerous safety studies showed that TT-232 is a molecule of low toxicity: no accumulation, allergic or mutagenic effects were seen. The most significant feature is that TT-232 does not affect the vital function or morphology of tissues, as most cytotoxic agents do.

In the present paper, we examined the activity of TT-232 in vitro and in vivo on P-388 mice lymphoid and HL-60 human promyelocytic tumor leukemia models. In vitro, the antiproliferative effect of the novel somatostatin structural derivative was very significant. It inhibited the proliferation of P-388 mice lymphoid and HL-60 human promyelocytic leukemia cells in the range of 46%-97% with treatment for 24 hours and 82%-100% with 48-hour treatment. The results of our experiments in vitro demonstrated that HL-60 tumor promyelocytic leukemia cells have similar sensitivity to treatment with TT-232 as P-388 mice lymphoid tumor cells. In vivo, we demonstrated the efficacy of TT-232 on the same leukemia tumor models transplanted in mice (P-388 mice lymphoid leukemia and HL-60 human promyelocytic leukemia). The tumor growth inhibitory effect of TT-232 on these leukemia tumor models proved to be significant. In vivo, with the P-388 mice tumor, the infusion of TT-232 by Alzet osmotic minipump resulted in 70%-80% tumor growth inhibition and 20% tumor-free survival. In the HL-60 human leukemia tumor model, long-term infusion treatment with TT-232 caused a 50% and 60% decrease in tumor volume and resulted in 20% and 40% tumor-free animals. Our experiments demonstrated that, in different leukemia tumor models, the application of high doses of TT-232 by infusion treatment results in a therapeutically significant tumor growth inhibition (Figure 3). We applied a long-term infusion of TT-232 using the Alzet osmotic minipumps (Model 2002) in order to maintain a low dose of the hormone in the circulation for a longer time period. The frequent and long-lasting repetition
of the novel somatostatin analog injection enhanced the therapeutic efficacy of the somatostatin analog, however, these serial injections represent significant stress to the animals and require precautions in terms of drug administration. To reduce and eliminate the above-mentioned problem, we used an Alzet osmotic minipump inserted s.c. Infusion from inserted Alzet minipumps maintains a constant drug level, resulting in a well-defined, consistent pattern of drug exposure throughout the period of drug administration. These studies suggest that TT-232 is a potent inhibitor of leukemia tumor in vitro and in vivo and suggest infusion treatment as a beneficial application in clinical practice.

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References