TT-232: a somatostatin structural derivative as a potent antitumor drug candidate

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TT-232 (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2) has been developed as an antitumor somatostatin analog. TT-232 has no growth hormone release inhibitory effect and does not inhibit the secretion of gastric acid. This analog induces apoptosis in and exerts pronounced antiproliferative effects on various human tumors (colon, pancreas, lymphoma, leukemia, melanoma, hepatoma) cell lines. The growth of human xenografts (prostate, breast carcinoma, lymphoma, melanoma) and animal tumors (colon-26, P-388, S-180, B16, MXT) was inhibited by TT-232 (dose range: 30–750 μg/kg/day) in 54–98% of cases. Continuous long-term infusion proved to be the most effective way of administration. TT-232 combined with decarbazine or etoposide treatment enhanced the antitumor activity of these drugs on human melanoma and lymphoma xenografts, respectively. 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. TT-232 may be a promising candidate in the therapy of human malignancies. *Anti-Cancer Drugs* 14:585–588 © 2003 Lippincott Williams & Wilkins.

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Introduction

Somatostatin is a naturally occurring peptide hormone which acts as an inhibitory factor at different target sites of the endocrine system and is an endogenous antiproliferative agent. This peptide hormone activates tyrosine phosphatases, thus indirectly inhibiting tyrosine kinases which are involved in the regulation of cell proliferation. In view of its widespread systemic action, the clinical use of somatostatin was evidently strongly dependent on new analogs selective in either hormonal or antitumor action. In the past decade a series of potent somatostatin analogs has been developed which also exert antitumor activity in certain hormone-dependent tumors. TT-232 has been developed as an antitumor somatostatin analog containing a five-residue ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2) and shows unique conformational characteristics compared to other somatostatin analogs [1–3].

Biological effects of TT-232

This analog had practically no growth hormone (GH) release inhibitory activity either in superfused rat pituitary cells or in rats *in vivo*. *In vivo* toxicology studies in mice showed that no death was observed even when this peptide was applied s.c. at a dose of 120 mg/kg body weight. TT-232 even at 120 mg/kg body weight had no effect on the number and the qualitative picture of the bone marrow cells. The acute i.v. LD_{50} values determined in NMRI mice and Wistar rats were 47 and 44 mg/kg, respectively. Histological studies of different organs from TT-232-treated mice did not show significant modification in the tissues. *In vitro* TT-232 did not inhibit the GH releasing hormone-induced GH release, and in *in vivo* assays in rats no decrease of initial serum GH levels was observed by TT-232 at the applied doses of 1 and 4 μg/100 g. TT-232 did not bind either to the pituitary or to the brain receptors and did not inhibit the secretion of gastric acid [4].

Effect of TT-232 on cell proliferation and cell death

TT-232 induces apoptosis (programmed cell death) in human colon (HT-29 and SW620), pancreatic (818), leukemia (K-562), melanoma (WM 938/B, M-1 and EP) and lymphoma (HT-58) tumor cell lines. The apoptotic index increased about 70 times in M-1 melanoma, 60 times in K-562 leukemia, 13 times in P818 pancreatic cells, 7 times in HT-29 human colon tumor cells and 3 times in PC-3 prostate tumor cells after 48 h incubation with 10 μg/ml of TT-232. In the case of WM melanoma and EP melanoma cells, 5 and 4 times increase of apoptotic index has been observed after 24 h of incubation with the same dose of TT-232 [4].

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TT-232 has a pronounced antiproliferative effect on differentiated and dedifferentiated, drug-sensitive and multidrug-resistant hepatocellular carcinoma cell lines.

TT-232 had antiproliferative activity in human pancreatic cancer cell lines and induced apoptotic cell death [5,6].

Proliferation of seven melanoma cell lines was tested *in vitro* with the methylene blue test. Two of these (205 and D10) were implanted into CB17-scid mice (four groups of eight mice). Animals received 30–150–750 μg/kg/day of TT-232 or saline. Those implanted with 205 cells received twice-daily s.c. injections, while D10-bearing mice were treated with s.c. osmotic mini-pumps. In addition, metabolism of TT-232 was studied in tissue homogenates and tested *in vitro*. TT-232 strongly inhibited proliferation of all cell lines *in vitro* and tumor growth *in vivo* [7]. Therapeutic efficacy of TT-232 was tested on different human tumor models (PC-3 prostate carcinoma, MDA-MB-231 breast carcinoma, HT-29 colon carcinoma, MCF-7 breast carcinoma, HT-18 melanoma and HL-60 promyelocytic leukemia), over 30 days (30 × qd) with s.c. intermittent injection and 14-day s.c. infusion treatment with application of an Alzet osmotic mini-pump (model 2002). The antitumor activity of TT-232 was evaluated on the basis of survival time and tumor growth inhibition. The tumor growth-inhibitory effect of TT-232 on human tumor xenografts proved to be significant, resulting in 30–80% decrease in tumor volume and 20–60% tumor-free animals. This antitumor efficacy of the novel somatostatin analog was observable in almost all tumors investigated [4,7–9]. The therapeutic effect of TT-232 was studied using different routes of administration and treatment schedules on various types of tumors of mice (S-180) sarcoma, P-388sc lymphoid leukemia, Colon-26 adenocarcinoma and MXT breast carcinoma. The results demonstrated the therapeutic efficacy of TT-232 on different rodent tumors; however; the tumor inhibitory effect was influenced by the dose, treatment schedule and sensitivity of the tumor against the somatostatin analog. The infusion treatment using implanted Alzet-type osmotic mini-pumps proved to be superior to both s.c. and i.v. infusion applied twice a day for 2 weeks. In the case of S-180 tumor, the infusion treatment of TT-232 for s.c. and i.v. implanted mini-pumps resulted in 77–100% tumor growth inhibition, and in 40–60% long-term and tumor-free survivors [9]. With the P-388sc tumor, the s.c. and i.v. infusion treatment resulted in 20–40% longer tumor-free survival and in 76–100% tumor growth inhibition of TT-232 in the treated mice. In the very aggressive Colon-26 and MXT mammary carcinoma, TT-232 treatment resulted in 71–75% tumor growth inhibition and increased the survival time by about 50% [8,9].

Table 1 summarizes the most important data on the tumor inhibitory effect of TT-232.

Effect of TT-232 combined with cytostatic compounds on tumor growth

TT-232, which inhibits the proliferation of various cell cultures and transplantable mouse tumors, was examined regarding its effect on human melanoma and lymphoma xenografts as a single treatment or in combination with dacarbazine (DTIC) and etoposide. TT-232 inhibited the growth of HT-18 melanoma xenografts, the dose of 5 mg/kg being the most effective. Combination of 1 mg/kg TT-232 with 30 or 60 mg/kg DTIC (daily administered) resulted in a stronger inhibitory effect compared to TT-232 or DTIC as a single modality. The antimetastatic effect of TT-232 treatment combined with DTIC was studied using the B16 mouse melanoma muscle–lung metastasis model. The number of lung metastases of B16 melanoma could be decreased by the daily administration of 1 mg/kg TT-232 or 60 mg/kg, but not of 30 mg/kg DTIC. TT-232 combined with 30 or 60 mg/kg DTIC

Table 1 Effect of TT-232 on various animal and human tumors

		Inhibition (%)	Reference
Tumors of animal origin			
S-180 sarcoma	14 × 15 μg/kg/day i.v.	70	9,17
	14 × 15 μg/kg/day i.v. osmotic mini-pump	98	
Colon-26 adenocarcinoma	14 × 15 μg/kg/day sc.	76	4,8
P-388 lymphoid leukemia	14 × 15 μg/kg/day sc.	80	8
MXT breast carcinoma	14 × 15 μg/kg/day i.v. osmotic mini-pump	74	8
B16 melanoma	19 × 750 μg/kg/day s.c	54	4
Tumors of human origin			
PC-3 prostate tumor	30 × 20 mg/kg s.c.	(80% cured)	4
MDA-MB-31 breast cancer	30 × 0.25 mg/kg s.c.	80	4
MCF-7 breast cancer	30 × 5 mg/kg s.c.	73	4
	14 × 0.3 mg/kg/day s.c.	67	
HT-18 melanoma	14 × 0.2 mg/kg/day s.c.	39	10 and unpublished
HT-168 melanoma	27 × 0.01 mg/kg i.v.	60	unpublished by Szende *et al.*
D10 melanoma	14 × 0.15 mg/kg i.v. osmotic mini-pump	54	7
HT-58 lymphoma	21 × 2.7 mg/kg	71	10 and unpublished

decreased the lung metastasis number significantly lower than the control. Nearly 50% growth inhibition of HT-58 lymphoma was achieved by daily treatment with 1 mg/kg TT-232. Etoposide at 5 mg/kg administered daily resulted in a similar effect. The combination of 1 mg/kg TT-232 and 5 mg/kg etoposide was significantly more effective than TT-232 or etoposide as a single treatment. The very strong tumor growth-inhibitory effect of 10 mg/kg etoposide could even be increased by combination with TT-232. These experimental data suggest that TT-232 may be an effective new tool in combination chemotherapy of malignant tumors like melanoma and lymphoma [10].

Summarizing these *in vivo* experiments, TT-232 reveals powerful anticancer activity in a wide range of various tumors of animal and human origin. Its effect in many experiments was not dose dependent (very sharp dose–response curve); in some cases lower doses were more effective. The mechanism behind this seemingly controversial issue has not been fully explored. There is growing evidence that a biphasic mode of action is the main reason. Optimal tumor growth-inhibiting effect has been observed when the drug was applied in continuous long-term (2–4 weeks) infusion. Combining TT-232 with chemotherapeutic agents acting on different mechanisms resulted in a strong synergistic inhibition of tumor growth and spread.

Studies on mode of action of TT-232

TT-232 induced signaling events in A431 cells, where a 4-h pre-incubation with the peptide irreversibly induced a cell death program, which involves DNA laddering and the appearance of shrunken nuclei, but is unrelated to somatostatin signaling. Early intracellular signals of TT-232 include a transient 2-fold activation of the extracellular signal-regulated kinase (ERK2), and a strong and sustained activation of the stress-activated protein kinases c-Jun NH_2-terminal kinase (JNK)/SAPK and p38MAPK. Blocking the signaling to ERK or p38MAPK activation had no effect on TT-232-induced cell killing. At the commitment time for inducing cell death TT-232 decreased epidermal growth factor (EGF) receptor tyrosine phosphorylation, and prevented EGF-induced events like cRaf-1 and ERK2 activation. Signaling to ERK activation by FCS, phorbol 12-myristate 13-acetate and platelet-derived growth factor was similarly blocked. Our data suggest that TT-232 triggers an apoptotic type of cell death, concomitant with a strong activation of JNK and a blockade of cellular ERK2 activation pathways [11].

A specifically tritium-labeled [^{3}H-Tyr3]TT-232 (30 Ci/mmol) has been synthesized and characterized to investigate the effect and the fate of this antitumor peptide on human colon tumor cells. ^{3}H-labeled TT-232 could be detected on the cell surface, on cytoplasmic membranes and also in the nuclei of HT-29 cells 1–6 h after the administration of 0.5 and 50 μg/ml [^{3}H]TT-232. Binding and internalization of TT-232 to human colon tumor cells at a relatively high dose provide further evidence for the existence of low-affinity somatostatin receptors in such cells, which might mediate the apoptosis-inducing effect [12].

Flow cytometric and electron microscopic immunocytochemical studies have been performed in HT-29 human colon tumor cells *in vitro* to determine and localize p86 Ku protein, which is a regulatory subunit of DNA-dependent kinase and a specific binding site for somatostatin. HT-29 cells contain p86 Ku, and the distribution between the cytoplasm and the nucleus is even. After administration of the somatostatin analogs Sandostatin and TT-232 to HT-29 cells, the p86 Ku content of the cytoplasmic compartment decreased in the first 4 h. An increase in the content of this protein in the nuclear compartment was observed at 1 h, followed by a decrease at 4 h after treatment. Quantitative differences between the two analogs have been observed in this respect [13].

In a further study, the effect of TT-232 on the PTPase activity in the SW620 human colon tumor cell line was investigated, where TT-232 caused a strong inhibition of cell proliferation. In response to TT-232 we found a rapid and sustained increase (5–30 min) in PTPase activity showing two maxima at 0.1 and 30 μM concentrations, respectively. During short-term incubation tyrosine kinase activity was much less affected by TT-232. TT-232-induced activation of PTPases may be an important early step in the signaling cascade in the inhibition of cell proliferation in colon carcinomas [14].

Apart from the ability to induce apoptosis, TT232 can trigger an alternative pathway that leads to cell cycle arrest in certain tumor cell systems. Pulse treatment with TT-232 blocks the cell cycle G_1/S transition irreversibly in A431 cells. Investigation of the TT-232 signaling pathway yielded results similar to those reported for somatostatin, although its affinity to the somatostatin receptor 1 is significantly reduced. Functional protein kinase C (PKC) δ as well as c-Src are necessary mediators of the TT-232 cytostatic effect and may characterize a signaling pathway that leads to cell cycle arrest [15,16].

The antiproliferative effect of TT-232 is partly mediated by SSTR receptors (SSTR1 and 5). The SSTR mediated signaling cascade activated by TT232 was fully elucidated. It activates a phosphorylation cascade (the MAP kinase pathway) and results in the induction of cyclin-dependent kinase inhibitors, causing cell cycle arrest. TT-232 not only binds to two of the five high-affinity receptors (SSTR1 and 5), but also enters the cells probably via passive transport or low-affinity receptors.

The apoptotic effect of TT232 is the result not only of cell cycle arrest, but the internalized TT232 binds to a still unidentified intracellular receptor which translocates it to the nucleus, where it causes apoptosis.

Interfering with the second messenger pathways by the interaction with these enzymes has an effect on cellular proliferation and apoptosis (programmed cell death) shown by apoptosis induction detectable both *in vitro* and *in vivo* [11].

Based on our present knowledge, TT-232 could have multiple synergistic antitumor effects:

(i) Inhibits tyrosine kinases, key enzymes of proliferative signaling, through interfering with the proliferative signaling cascades.
(ii) Via SSTR receptors, activates cell cycle inhibitors inducing cell cycle arrest.
(iii) Interacts with an intracellular receptor and an enzyme involved in glycolysis, and causes the translocation of this enzyme to the nucleus, thus causing apoptosis.

The fact that the optimal growth-inhibitory effect was reached on many tumors via infusion indicates that a continuous drug concentration is needed in order to replace inactivate substance or molecules that have left the intracellular space.

In summary, the results of both *in vitro* and *in vivo* tests have indicated that the modes of action of the antineoplastic effect of TT-232 differ from those of most known anticancer agents. Specifically, the interaction of TT-232 with enzymes that regulate cell proliferation play a substantial role in the antineoplastic effect. This is manifested by apoptosis induction detectable both *in vitro* and *in vivo*. The anticancer effect of TT-232 in various tumor models caused by interfering with specific signaling pathways involved in cell proliferation results not only in effective tumor growth and spread inhibition, but in a highly significant improvement in survival.

Conclusion

The somatostatin analog TT-232 decreased cell proliferation activity and enhanced apoptosis in various *in vitro* and *in vivo*, experimental animal and human tumor systems. Because of its strong antitumor activity and minor side-effects, this compound may be a promising candidate in the therapy of human malignancies.

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References

1 Kéri Gy, Mező I, Horváth A, Vadász Zs, Balogh Á, Idei M, *et al.* Novel somatostatin analogs with tyrosine kinase inhibitory and antitumor activity. *Biochem Biophys Res Commun* 1993; **191**:681–687.
2 Kéri Gy, Mező I, Vadász Zs, Horváth A, Idei M, Vántus T, *et al.* Structure–activity relationship studies of novel somatostatin analogues with antitumor activity. *Peptide Res* 1993; **6**:281–288.
3 Jaspers H, Horváth A, Mező I, Kéri Gy, Van Binst G. Conformational study of a series of somatostatin analogues with antitumor and/or GH inhibitory activity. *Int J Peptide Protein Res* 1994; **43**:271–276.
4 Kéri Gy, Érchegyi J, Horváth A, Mező I, Idei M, Vántus T, *et al.* Tumor-selective somatostatin analog (TT-232) with strong *in vitro* and *in vivo* antitumor activity. *Proc Natl Acad Sci USA* 1996; **93**:12513–12518.
5 Diaconu C-C, Szathmári M, Kéri Gy, Venetiaer A. Programmed cell death (apoptosis) is induced in both drug-sensitive and multidrug-resistant hepatoma cells by somatostatin analog TT-232. *Br J Cancer* 1999; **80**: 432–438.
6 Lee U, Hosotani R, Wada M, Doi R, Koshiba T, Fujimoto K, *et al.* Antiproliferative activity induced by the somatostatin analogue TT-232, in human pancreatic cancer cells. *Eur J Cancer* 2002; **38**:1526–1534.
7 Schwab ER, Froidevaux S, Paku S, Tejeda M, Szende B, Pap A, *et al.* Antiproliferative efficacy of the somatostatin analogue TT-232, in human melanoma cells and tumors. *Anticancer Res* 2001; **21**:71–75.
8 Tejeda M, Gaál D, Csuka O, Ullrich A, Schwab RE, Pap Á, Horváth A, *et al.* Antitumor efficacy of TT-232, the antitumor effect of the somatostatin analogue TT-232 depends on the treatment regimen. *Cancer Detect Prevent* 2003; **27**:155–162.
9 Tejeda M, Gaal D, Schwab RE, Pap A, Szűts T, Kéri Gy. Influence of various administration routes on the antitumor efficacy of TT-232, a novel somatostatin analog. *Anticancer Res* 2000; **20**:1023–1028.
10 Szende B, Horváth A, Bökönyi G, Kéri Gy. Effect of a novel somatostatin analogue combined with cytotoxic drugs on human tumour xenografts and metastasis of B16 melanoma. *Br J Cancer* 2003; **88**:132–136.
11 Vántus T, Kéri Gy, Krivickiene Z, Valius M, Steták A, Keppens S, *et al.* The somatostatin analogue TT-232 induces apoptosis in A431 cells. Sustained activation of stress-activated kinases and inhibition of signaling to extracellular signal-regulated kinases. *Cell Signal* 2001; **13**:717–725.
12 Szegedi Zs, Takács J, Szende B, Vadász Zs, Horváth A, Gulyás É, *et al.* A specifically radiolabeled somatostatin analog with strong anti-tumor activity induces apoptosis and accumulates in the cytosol and the nucleus of HT29 human colon carcinoma cells. *Endocrine* 1999; **12**:25–34.
13 Tóvári J, Szende B, Bocsi J, Falaschi A, Simoncsits A, Pongor S, *et al.* Somatostatin analogs induced translocation of Ku-86 autoantigen from the cytosol to the nucleus in colon-tumor cells. *Cell Signal* 1998; **10**:277–282.
14 Vántus T, Csermely P, Mezõ I, Teplán I, Kéri Gy. The tumor-selective somatostatin analog, TT-232 induces biphasic activation of phosphotyrosine phosphatase activity in human colon tumor cell line, SW620. *Tumor Biol* 1995; **16**:261–267.
15 Steták A, Lankenau A, Vántus T, Csermely P, Ullrich A, Kéri Gy. The antitumor somatostatin analogue TT-232 induced cell cycle arrest through PKCδ and c-Src. *Biochem Biophys Res Commun* 2001; **108**:25–33.
16 Steták A, Csermely P, Ullrich A, Kéri Gy. Physical and functional interactions between protein tyrosine phosphatase δ, PI 3-kinase, and PKCδ. *Biochem Biophys Res Commun* 2001; **288**:564–572.
17 Tejeda M, Gaal D, Schwab RE, Pap A, Kéri Gy. *In vivo* antitumor activity of TT-232 a novel somatostatin analog. *Anticancer Res* 1999; **19**:3265–3268.