Evaluation of the Antitumor Efficacy of the Somatostatin Structural Derivative TT-232 on Different Tumor Models

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Abstract. *The antitumor effects of the somatostatin structural derivative TT-232 in different rodent and xenograft tumor models are summarized in this report. 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. The effects of TT-232 by different routes of administration and treatment schedules were studied in various types of rodent and human xenograft tumor models. In the rodent tumor models S-180 sarcoma and P-388 lymphoid leukemia tumor the infusion treatment resulted in 76%-100% tumor growth inhibition and in 20%-60% of the mice being long-term and tumor-free survivors. In the aggressive C-26 colon carcinoma and MXT breast carcinoma, the TT-232 treatments resulted in 71%-75% tumor growth inhibition and an approximately 50% increased survival time. 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 in 20%-40% tumor-free animals. This 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. Our results suggested that TT-232 is an effective and promising antitumor agent.*

Somatostatin, a natural tetradecapeptide hormone, inhibits the release of the growth hormone and various endocrine secretions (*i.e.*, glucagon, insulin, gastrin). 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 (1-3). The natural hormone was shown to act as an inhibitory factor at different target sites of the endocrine system, to reduce portal pressure and to exhibit a marked antiproliferative activity on various tumor cells, both *in vitro* and *in vivo* (4). Because of the short half-life of the natural compound, analogs of somatostatin were designed to enable the clinical application of the peptide (5-8).

A new potent tumor-selective somatostatin structural derivative (TT-232), with a 5-residue ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2), was developed and reported by us (9-13). TT-232 was shown to inhibit proliferation and to induce apoptosis both *in vitro* and *in vivo* in various types of tumor cells. The antitumor activity of TT-232 has been found to be associated with the 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, which paralleled apoptosis, by both biochemical and morphological parameters (14-17). The TT-232-induced inhibition of the growth-promoting tyrosine kinase signal could be coupled to the inhibition of the regular cell cycle ending in apoptosis. 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 non-apoptotic programmed cell death (18, 19). TT-232 has been shown to have unique conformational characteristics, selective binding properties to the first and fourth subtypes of somatostatin receptors (SSTR1 and 4) and to the M2 isoform of the intracellular receptor, pyruvate kinase. Its mechanism of action of TT-232 follows the new era of molecular medicine called signal-transduction therapy, where "internal communication" of cells is corrected without interfering with the basic cell functions and machinery. Kinases and phosphatases have been identified as important

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target molecules of signal transduction therapy. The involved molecular mechanism has been linked to both short-term activation of the intracellular tyrosine phosphatases and the long-term inhibition of tyrosine kinases. Short-term (30 min) exposure of cells to TT-232 activates the SSTR receptors (primarily the SSTR1), which leads to irreversible cell cycle arrest in G_1/S, followed by secondary induction of apoptosis (20). In contrast, continuous incubation with TT-232 leads to direct induction of active cell death, independently of SSTR-mediated signaling (21). The mechanism of action and the signaling cascade of TT-232 have been fully elucidated (22-27).

Here, the therapeutic efficacy of TT-232 was studied on the basis of survival and tumor growth inhibition, using various rodent and human tumor models and applying different administration routes and treatment schedules.

Materials and Methods

Administration route and treatment schedule of TT-232. The antitumor effects of the somatostatin structural derivative TT-232 were studied on different rodent and human xenograft tumor models. In the rodent tumor models, the therapeutic efficacy of TT-232, when treatments were started on day 1 after tumor transplantation or after tumor development was investigated. The *in vivo* responsiveness was tested in a wide dose range (5-500 μg/kg). On the basis of our previous experiments (28-35), the optimum dose of injected TT-232 was determined to be 15 μg/kg twice a day. This dose equals to 0.6 μg per day by infusion therapy. The therapeutic effects of the TT-232 were examined in different rodent tumor models using subcutaneous (*s.c.*) and intravenous (*i.v.*) injections over 14 days and 7- and 14-day *s.c.* or *i.v.* infusion treatments employing an Alzet osmotic minipump (model 2001 or 2002). When 28 days of TT-232 infusion were administrated successively, two Alzet mp (model 2002) were utilized.

In the case of human tumor xenograft models, the injected doses were 0.25-20.0 mg/kg of TT-232 body weight, while the infusion doses by model 2002 Alzet mp were 3.0-12.0 μg/day. Relatively low doses of TT-232 were applied, although our *in vitro* data showed that, for the dramatic antiproliferative- and apoptosis-inducing effect, a critical dose, depending of the cell type, had to be reached. TT-232 had strong *in vivo* activity even at relatively low doses, indicating the likelihood of reaching the critical threshold for apoptosis induction with higher doses. Administration of TT-232 with the mp was carried out as instructed by the manufacturer (36, 37). The animals used in these experiments were cared for according to the "Guiding Principles for the Care and Use of Animals" based upon the Helsinki's declaration and they were approved by the local ethical committee. The antitumor activity of TT-232 was evaluated on the basis of survival time and tumor growth inhibition (38).

The materials and methods data of tumor models are described in our previous publications: S-180 sarcoma (30); P-388 mice and HL-60 human leukemia models (39); C-26 adenocarcinoma, MXT Mammary carcinoma (32); PC-3 prostate carcinoma, MDA-MB-231 (ER-) and MCF-7 (ER+) breast carcinoma, HT-29 colon carcinoma, HT-18 melanoma, HL-60 promyelocytic leukemia (33).

Results and Discussion

The somatostatin structural derivative, TT-232, has a special 5-residue ring structure (D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2) 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 the rat pituitary or the cortex, where all the known somatostatin receptor subtypes are expressed. 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.*- or *i.v.*-inserted mp on different types of transplantable rodent (S-180 sarcoma, P-388sc lymphoid leukemia, C-26 colon carcinoma and MXT breast carcinoma) and human tumor xenograft models (PC-3 prostate carcinoma, MDA-MB-231 (ER–) and MCF-7 (ER+) breast carcinoma, HT-29 colon carcinoma, HT-18 melanoma and HL-60 promyelocytic leukemia). Long-term infusion of TT-232 *via* mp was used in order to maintain a low dose of the peptide in the circulation for a longer time period. A significant effect of TT-232 on tumor growth inhibition and long-term survival was observed after repeated injections or infusion treatment mp. Our results and the data of other authors (30-43) demonstrated that the therapeutic doses given by infusion achieved significantly greater reductions in tumor size than identical doses given by either of the injection schedules. However, the tumor inhibitory effect was also dependent on the sensitivity of the tumor to this somatostatin structural derivative. On the basis of our previous experiments (44), the optimum injected dose of TT-232 was found to be 15 μg/kg twice a day, equaling 0.6 μg per day by infusion therapy. In terms of antitumor therapy, the best results were achieved when TT-232 was applied at an infused dose of 15 μg/day regardless of when the treatment had been started. In the case of S-180 sarcoma and P-388sc lymphoid leukemia tumor, the infusion treatments produced 67%-100% inhibition of tumor growth and resulted in 20%-60% long-term and tumor-free survivors. In the C-26 colon adenocarcinoma and MXT breast carcinoma tumor-bearing mice, the infusion treatments drastically inhibited the tumor growth and increased the survival time by about 52%-75%. Our previous results on S-180 sarcoma tumor model were in good agreement with the present findings and demonstrated the efficacy of TT-232 on various aggressive animal tumor models (Figures 1, 2 and Table I). The tumor growth inhibitory effect of TT-232 on human xenograft models proved to be significant. The results indicated that the daily (0.25-20.0 mg/kg doses) *s.c.* injections caused a 15%-80% decrease in the tumor volume and resulted in 25%-60% tumor-free animals. The best results were obtained with application of high doses of TT-232 over

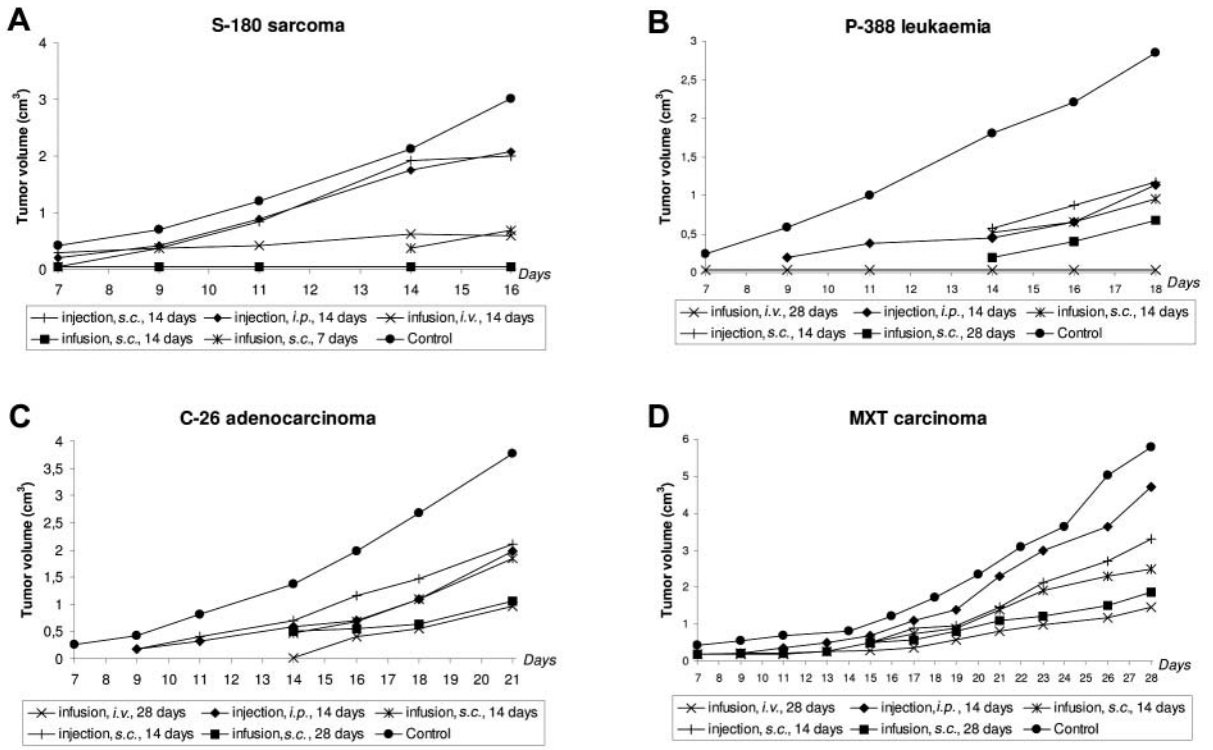

Figure 1. *Tumor growth-inhibitory effect of TT-232 applied by various administration routes in different rodent tumor models. The TT-232 treatments were started on day 1 after tumor transplantation.*

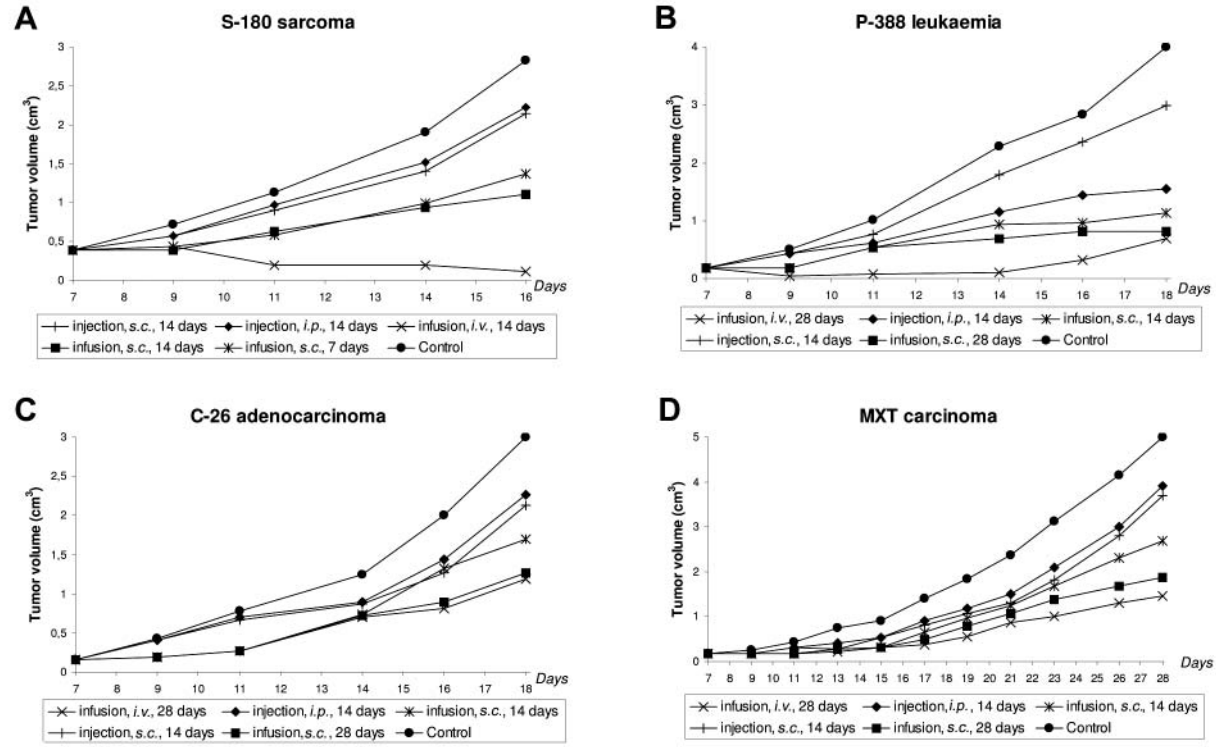

Figure 2. *Tumor growth-inhibitory effect of TT-232 by various administration routes in different rodent tumor models. The TT-232 treatments were started after the development of the tumor.*

Table I. *Influence of the different routes of administration and schedules on the therapeutic effects of TT-232 in various rodent tumors.*

Tumor type	Treatment type	Days	Route	Maximun tumor inhibition (%)	Tumor-free survivors	Treatments started
S-180 sarcoma	injection	14	*i.p.*, *s.c.*	32%	30%	Treatments started on day 1 after tumor transplantation
	infusion	7	*s.c.* mp	77%	0%	
		14	*s.c.* mp	100%	60%	
		14	*i.v.* mp	80%	40%	
P-388 lymphoid leukemia	injection	14	*i.p.*, *s.c.*	59%	0%	
	infusion	14	*s.c.* mp	67%	0%	
		28	*s.c.* mp	76%	20%	
		28	*i.v.* mp	100%	40%	
C-26 colon adenocarcinoma	injection	14	*i.p.*, *s.c.*	48%	0%	
	infusion	14	*s.c.* mp	52%	0%	
		28	*s.c.*, *i.v.* mp	75%	0%	
MXT breast carcinoma	injection	14	*s.c.*	39%	0%	
	infusion	28	*s.c.*, *i.v.* mp	71%	0%	Treatments started after the development of tumor
S-180 sarcoma	injection	14	*i.p.*, *s.c.*	24%	0%	
	infusion	14	*s.c.* mp	61%	40%	
		14	*i.v.* mp	96%	40%	
P-388 lymphoid leukemia	injection	14	*i.p.*	61%	0%	
	infusion	28	*s.c.*, *i.v.* mp	80%	20%	
C-26 colon adenocarcinoma	injection	14	*i.p.*, *s.c.*	29%	0%	
	infusion	28	*s.c.*, *i.v.* mp	60%	0%	
MXT breast carcinoma	injection	14	*i.p.*, *s.c.*	26%	0%	
	infusion	28	*s.c.*, *i.v.* mp	70%	0%	

i.p.: intraperitoneal; *s.c.:* subcutaneous; *i.v.:* intravenous.

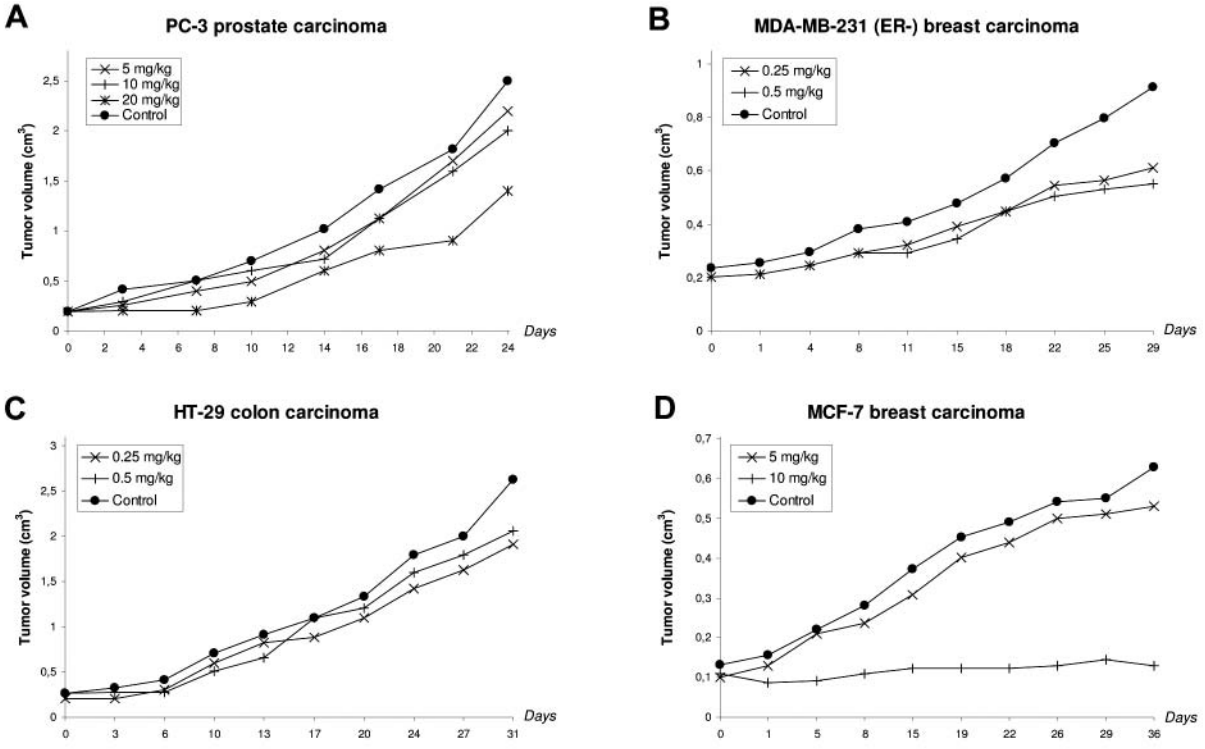

Figure 3. *Effect of the antitumor efficacy of TT-232 on different human tumor xenografts.*

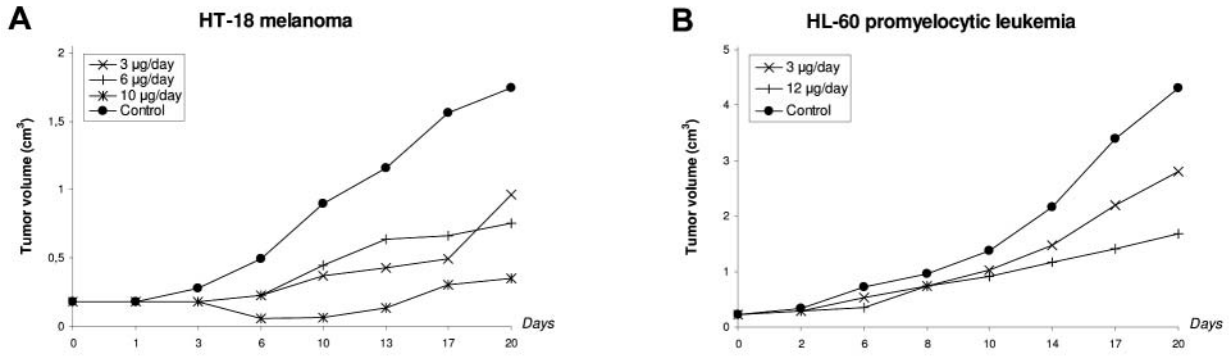

Figure 4. *Effect of the antitumor efficacy of TT-232 on various human tumor xenografts.*

Table II. *Influence of the different doses and modes of administration on the therapeutic effect of TT-232 in various xenografted human tumor-bearing mice.*

Treatment type	Tumor type	Dose	Route	Days	Maximun tumor inhibition (%)	Tumor-free survivors
Injection	PC-3 prostate carcinoma	5 mg/kg	*s.c.*	30	15%	0%
		10 mg/kg	*s.c.*	30	21%	40%
		20 mg/kg	*s.c.*	30	40%	60%
	MDA-MB-231 breast carcinoma	0.25 mg/kg	*s.c.*	30	33%	25%
		0.50 mg/kg	*s.c.*	30	39%	37%
	HT-29 colon carcinoma	0.25 mg/kg	*s.c.*	30	27%	0%
		0.5 mg/kg	*s.c.*	30	21%	0%
	MCF-7 breast carcinoma	5 mg/kg	*s.c.*	30	18%	12.5%
		10 mg/kg	*s.c.*	30	80%	25%
Infusion	HT-18 melanoma	3 µg/day	*s.c.* mp	14	44%	40%
		6 µg/day	*s.c.* mp	14	57%	40%
		10 µg/day	*s.c.* mp	14	80%	20%
	HL-60 promelocytic leukemia	3 µg/day	*s.c.* mp	14	34%	40%
		12 µg/day	*s.c.* mp	14	60%	20%

s.c.: subcutaneous.

30 days (30 xqd) and *s.c.* intermittent injection treatments (PC-3 prostate carcinoma, MDA-MB-231 (ER–) and MCF-7 (ER+) breast carcinoma). After the intermittent injections and *s.c.* treatments, infusion treatment of TT-232 with Alzet mp was applied to increase its tumor efficacy. In these experiments, 3.0-12.0 µg/day of TT-232 was applied *via* the 2002 mp. In the case of long-term infusion, TT-232 caused 34%-80% decrease in the HT-18 melanoma and HL-60 promyelocytic leukemia tumor volumes and resulted in 20%-40% tumor-free animals (Figures 3, 4 and Table II).

Our experiments demonstrated that in different human tumor models, much better results were obtained with the application of low doses of TT-232 in infusion treatment, than by its application in high doses and by *s.c.* intermittent injection treatments. After comparing the total amounts of TT-232 applied by the different administration routes, it became evident that the infusion route of treatment significantly increased (by two orders of magnitude) the specific activity of TT-232. Our comparative experiments confirmed that continuous treatments and long-term administration were associated with the best treatment response in all the *in vivo* models studied. However, minor differences were detected between the *s.c.* and *i.v.* administration routes, which warrant further investigations. By extrapolating the results to clinical application, continuous infusion therapy is the most promising alternative. The long-term infusion of TT-232 by mp was to maintain a low-dose of the hormone in the circulation for a longer time-period. Our results demonstrated the antitumor efficacy of TT-232 on different rodent and

human tumor xenograft models, however, this was influenced by the dose, the treatment schedule and by the sensitivity of the tumor to TT-232. The frequent and long-term repetition of TT-232 injections enhanced its therapeutic efficacy although serial injections caused significant stress to the animals and required precautions in administration. To reduce and eliminate this problem, the Alzet osmotic mp were inserted *s.c.*, maintaining a constant drug level and resulting in a well-defined, consistent pattern of drug exposure, which indicates the potential benefits of TT-232 in clinical practice. Continuous infusion *via* Alzet mp is feasible only when the administered drug is stable throughout the delivery period. The stability of TT-232, both in solid (lyophilized) form and in aqueous solution during storage at different temperatures was investigated. Samples stored for various time-periods were analyzed for TT-232 content, as well as for degradation products, using HPLC methods (45).

The programmed infusion of TT-232 may be a promising method. Development of the optimum treatment schedule and the significant sensitivity to TT-232 in all tested rodent and human tumors represent promising data for human clinical trials. Our results suggest that TT-232 is a promising new antitumor agent in cancer chemotherapy.

Acknowledgements

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