Abstract. The tumor growth inhibitory efficacy of the somatostatin structural derivative TT-232 was studied using different routes of administration and treatment schedules in various human tumor models. TT-232, containing a five-residue ring structure, has a strong antitumor activity both in vitro and in vivo. The antineoplastic activity of TT-232 has been found to be associated with the induction of programmed cell death in tumor cells, resulting in highly-selective elimination of the neoplastic tissue. The study compared the antitumor efficacy of TT-232 in various long-term administration routes; the intermittent (injection) versus continuous (infusion) treatment via subcutaneously-inserted Alzet osmotic minipumps in different human tumor models: T-47/D human breast carcinoma and A-431 human epidermoid carcinoma. Treatment with TT-232 started after disease development. The antitumor activity of TT-232 was evaluated on the basis of the tumor growth inhibition. In the case of T-47/D human breast carcinoma, the intermittent treatment resulted in 23%-26% and the infusion treatment resulted in 48%-53% tumor growth inhibition. The tumor growth inhibitory effect of TT-232 on A-431 human epidermoid carcinoma tumor resulted in 35%-43% (intermittent treatment) and 70%-74% (continuous treatment) decreases in tumor volume. This antitumor efficacy of TT-232 was observed in the two human tumors investigated. In this study, the route of infusion was shown to increase drug efficacy relative to conventional delivery methods. The results suggest that TT-232 is an effective and promising antitumor agent.

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. TT-232 is an endogenous hormone with significant antiproliferative properties. In contrast to the parent hormone and the "traditional" somatostatin analogs, this compound has strong and selective growth-inhibitory potential without the wide-ranging endocrine side-effects. TT-232 has been shown to inhibit proliferation and induce cell death (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 the short-term activation of intracellular tyrosine phosphatases and long-term inhibition of tyrosine kinases (1). 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 the secondary induction of apoptosis (2). In contrast, continuous incubation with TT-232 led to direct induction of active cell death independently of SSTR-mediated signaling (3). Similarly to natural somatostatin (4), TT-232 had an early transient activator effect on the ERK/MAPK pathway, which led to the induction of the cyclin-dependent kinase inhibitor p21cip1/waf1, causing irreversible cell cycle arrest in the G1/S-phase (5). The mechanism of action and the signaling cascade of TT-232 have been fully elucidated (6-10). The present study was undertaken to define the optimal route for TT-232 administration and to evaluate the antitumor efficacy of such treatment in two human tumor models: T-47/D human breast carcinoma and A-431 human epidermoid carcinoma tumor. Continuous (infusion) administration of TT-232 significantly inhibited the growth of the tumor when compared with intraperitoneal (i.p.) and subcutaneous (s.c.) intermittent (injection) treatments.
Materials and Methods

**Compound.** TT-232 (a somatostatin analog, a cyclic heptapeptide) was dissolved in buffer solution (pH=4.1) containing 0.1 M acetic acid, 0.1 M sodium acetate and mannit diluted with distilled water. The solution of TT-232 proved to be stable at 37°C over 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). Female (22-24 g) immuno-compromised (SCID) mice were used for these experiments. 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. The animals used in these studies were cared for according to the “Guiding Principles for the Care and Use of Animals”, based upon the Helsinki declaration, which were approved by the local ethical committee.

**Tumor cells.** T-47/D human breast carcinoma cells and A-431 human epidermoid carcinoma cells (obtained from the American Type Culture Collection (ATCC), Rockville, MD, USA) were used.

**Osmotic minipump.** The Alzet osmotic minipump (Model 2002, delivering 1.0 μl/hour for 14 days) was obtained from the Alza Corporation (Palo Alto, CA, USA). 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 minipumps was carried out as instructed by the manufacturer (11, 12). The animals were anesthetized by sodium-pentobarbital (NEMBUTAL® Abbot Lab., Ceva, Paris, France) at a dose of 50 mg/kg, i.p. 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 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 (13).

**Administration route and treatment schedule of TT-232.** On the basis of our previous experiments (14-20), it was determined that the optimum dose of TT-232 was 15 μg/kg twice a day in the case of injection treatment. This injection dose equals 0.6 mg per day of the infusion therapy. In the present experiments, the therapeutic effects of TT-232 in different human tumor models 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.

**The transplantation of tumors.** An optimal fragment (2-5x10² mg) of the tumor was transplanted s.c. into the intrascapular region of the mice. Treatment with TT-232 started after tumor development. In all cases, normal saline was used as a control. Ratio of the volume/body weight: 0.1 ml/10 g.

**Evaluation, statistical analysis.** The animals were weighed and the tumor volumes were measured with a microcaliper on every second or third day. The tumor volume was calculated by the following formula: \( V = \frac{4}{3} \pi L \times D^2 \) (\( V \) = tumor volume, \( L \) = longest diameter, \( D \) = diameter perpendicular to \( L \)). The experimental data were subjected to computerized statistical analysis of variance with the Student-Newman-Keuls test. Statistical significance was accepted at the p<0.05 level (21).
Results

The antitumor efficacy of TT-232 on T-47/D human breast carcinoma tumor. The tumor inhibitory effects of TT-232, via injection and infusion treatment, on the T-47/D human breast carcinoma model were investigated. Treatments were started 13 days after development of the tumor. When TT-232 was applied at a dose of 15 mg/kg by s.c. and i.p. injection for 30 days (30xqd), a moderate (23% and 26%) tumor growth-inhibitory effect was observed. On the basis of tumor growth curves, a significant inhibitory activity of TT-232 was observed following long-term infusion using Alzet 2002 tip osmotic minipumps implanted s.c. TT-232 administered via the s.c. minipump for 14 days evoked a significant (48%) tumor growth-inhibitory effect. In the case of the s.c.-implanted pumps used for 28 days, a 53% tumor growth-inhibitory effect was achieved. (Figures 1A, 2).

The antitumor effect of TT-232 on A-431 human epidermoid carcinoma tumor. The antitumor activities of TT-232 through intermittent (injection) and continuous (infusion) treatments were compared. The treatments were started 13 days after the development of the tumor. Figures 1B and 2 demonstrate the tumor inhibitory effect of TT-232 on A-431 human epidermoid carcinoma tumor using different routes of administration. When TT-232 was given at a dose of 15 mg/kg for 30 days (30xqd), the tumor growth-inhibitory effect was 35% (s.c. injection) and 43% (i.p. injection), respectively. The s.c. infusion treatments for 14- and 28-day periods, respectively, resulted in 70% and 74% tumor inhibitory effects.
Discussion

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 (Model 2002), on different human tumor models (T-47/D human breast carcinoma and A-431 human epidermoid carcinoma). A long-term infusion of TT-232 with the Alzet minipumps was used in order to maintain a low dose of the peptide in the circulation for a longer time-period. The results and data of previous experiments (22-25) 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. 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 the T-47/D human breast carcinoma tumor model, the influence of different administration routes and treatment schedules on the therapeutic effect of TT-232 was compared and expressed in terms of tumor growth inhibition. When TT-232 was given as a repeated dose of 15 μg/kg for 30 days (30xqd), the tumor growth-inhibitory effects were 25% (s.c. injection) and 26% (i.p. injection). The s.c. infusion treatment for 14 and 28 days with the application of Alzet osmotic minipumps resulted in 48% (p<0.005) and 53% (p<0.005) tumor-inhibitory effects. In the case of the A-431 human epidermoid carcinoma tumor model, when TT-232 was given at a dose of 15 μg/kg by s.c. and i.p. injection for 30 days (30xqd), the tumor growth-inhibitory effects were 35% (p<0.01) and 43% (p<0.01). When TT-232 was administered via a s.c. minipump for 14 days, TT-232 induced a significant (70%, p<0.005) tumor growth-inhibitory effect. A significant (74%, p<0.005) growth-inhibitory effect was obtained by the s.c. infusion for 28 days. The results of these experiments demonstrated that, in different human tumor models, much better results were obtained with the application of low doses of TT-232 in continuous (infusion) treatment, rather the application in high doses or i.p. and s.c. intermittent (injection) treatments. If the total amounts of TT-232 applied by the different administration routes (injection and infusion) were compared, it would be 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 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 minipumps 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. 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.

References