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Antiproliferative activity induced by the somatostatin analogue, TT-232, in human pancreatic cancer cells

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Abstract

Somatostatin analogues have been developed as antiproliferative agents, but their administration as general antitumour agents is limited, mainly because of the wide distribution of somatostatin receptors throughout the human body. TT-232, a new somatostatin structural analogue, was reported to have tumour-selective antiproliferative activity without an antisecretory action. We examined whether TT-232 had antiproliferative activity in human pancreatic cancer cell lines, and compared its antiproliferative activity with that of RC-160 and other TT-232 derivatives. TT-232 inhibited the growth of all of the cell lines used in this study and induced apoptotic cell death. RC-160 showed no such growth inhibition. TT-232 also inhibited tumour formation in a xenograft model. A competitive binding assay was performed using the cell membrane fraction and ^{111}In-DTPA-TT-232 in order to show the existence of a specific binding site on the cells. A specific binding site was detected in MIAPaCa-2 cells. It has been shown that the activation of protein tyrosine phosphatase (PTPase) is one of the main intracellular pathways responsible for somatostatinergic inhibition of cell growth. We found a significant PTPase stimulation after TT-232 administration using an immunoblot analysis assessing the level of protein tyrosine phosphorylation, and also a direct measurement of the PTPase activity. We also demonstrated that PTPase stimulation by TT-232 was involved in its antiproliferative activity as this activity was reversed by the addition of sodium orthovanadate, a PTPase inhibitor. Our results indicate that TT-232 could be a potentially useful therapeutic agent if these data are translated into clinical practice. © 2002 Published by Elsevier Science Ltd.

Keywords: Somatostatin; TT-232; Pancreatic cancer; Cell line; Growth inhibition

1. Introduction

Somatostatin is a tetradecapeptide that is involved in a variety of biological processes including inhibition of endocrine secretion and cell proliferation [1,2]. Somatostatin exerts its direct antiproliferative activity through specific cell surface receptors [1–3]. Five somatostatin receptor subtypes (SSTR1–SSTR5) and one splice variant have been cloned from humans, mice and rats [4–9]. The distribution of the receptor subtypes varies among organs in the human body or among the different cell lines. Somatostatin-14 and somatostatin-28 have a certain affinity for all of the somatostatin receptor subtypes. Somatostatin receptor subtype 2 (SSTR2) is widely accepted as a major subtype that mediates the antiproliferative activity of somatostatin. It is not exactly determined what kind of SSTRs mediate the antiproliferative activity of somatostatin, and what kind of effector molecules are related to the antiproliferative activity. In an *in vitro* study, the stimulation of protein tyrosine phosphatase (PTPase) activity was reported to be an essential step in mediating the antiproliferative activity of somatostatin [10,11], and induction of the apoptosis was also important [12,13].

In recent years, several somatostatin analogues have been developed for the purpose of clinical appliance.

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For example, Octreotide and RC-160 were developed as long-acting somatostatin analogues, and were reported to have a stronger affinity to SSTR2 and SSTR5 than the other SSTRs. These analogues were used in the diagnosis and/or treatment of tumours that expressed SSTR2 substantially on their cell surface, such as pituitary adenomas and neuroendocrine tumours of the gut. Clinical studies, however, revealed that somatostatin analogue therapy did not produce an adequate clinical response to patients suffering from advanced colon and pancreatic cancers [14,15]. This was thought to be due to a lack of somatostatin binding sites, especially SSTR2, in these tumours. Buscail and colleagues investigated the gene expression of the five SSTRs in 47 human normal and cancerous tissues or cell lines of pancreatic and colorectal origin, and reported that mRNA of the SSTRs was detected in 98% of samples, with more than two mRNA subtypes being expressed in 55% of cases, and *sstr1*, *sstr4*, and *sstr5* were heterogeneously expressed in both normal and cancerous tissues; *sstr3* was rarely or not expressed; *sstr2* was present in normal pancreatic tissues, but was absent in exocrine pancreatic carcinomas and their metastasis [16]. Fisher and colleagues examined mRNA expression of the somatostatin receptor subtypes and receptor binding in nine human pancreatic carcinoma cell lines, and found that only the MIAPaCa-2 cells had functional cell surface somatostatin receptors despite the fact that *sstr1*, *sstr2*, and *sstr5* were transcribed in the majority of the cell lines. They speculated that the number of cell surface receptors might be too low to be detected, or that there was a post-transcriptional defect in the tumour cells [17]. Furthermore, Delesque and colleagues demonstrated that pancreatic cancer cells without *sstr2* gene expression showed a reversal of tumorigenicity when transfected with human *sstr2* cDNA, and suggested that the loss of *sstr2* contributes to the malignancy of human pancreatic cancers [18]. Taking these facts into consideration, it may be postulated that somatostatin analogues that have a high affinity to SSTR2 are not effective in growth reduction against human pancreatic cancer. In fact, octreotide improved the symptoms of patients with advanced pancreatic cancers, but showed little antitumour effect.

Clinical use of somatostatin analogues as general antitumour agents is limited because of their antisecretory side-effects and sparse expression of the receptors mediating antiproliferative activity on target cells [3,19,20]. Keri G. and colleagues developed TT-232 as a novel somatostatin analogue with a unique five-residue ring structure, and demonstrated potent and tumour-selective antiproliferative activity without an antisecretory action [21]. In this study, we examined whether TT-232 had antiproliferative activities on human pancreatic cancer cell lines, and compared the antiproliferative activities of TT-232 with RC-160, which has a high affinity to SSTR2. In order to clarify the mechanism of TT-232, we tried to demonstrate apoptosis induction and specific receptor binding of TT-232, and examined whether PTPase stimulation was related to the antiproliferative activity of TT-232 using an immunoblot analysis for phosphorylated proteins with tyrosine residues and direct PTPase measurement. We also examined whether the antiproliferative activity of TT-232 was inhibited by sodium orthovanadate, a PTPase inhibitor. Furthermore, the antitumour effect of TT-232 was examined in a xenograft model.

2. Materials and methods

2.1. Cells

Human pancreatic cancer cell lines, PANC-1, AsPC-1, CAPAN-2, CFPAC-1 and MIAPaCa-2, were purchased from the American Type Culture Collection. PANC-1 cells were maintained as a monolayer culture in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humid atmosphere of 5% CO_2/95% air. CAPAN-2, CFPAC-1 and MIAPaCa-2 cells were maintained in McCoy's 5A Medium, Iscove's Modified Eagle Medium and Minimum Essential Medium, respectively, supplemented with 10% fetal bovine serum under the same conditions as for PANC-1 cells. AsPC-1 cells were maintained in Roswell Park Memorial Institute (RPMI)1640 Medium supplemented with 20% fetal bovine serum and under the same conditions as for PANC-1 cells. All experiments were performed during the exponential growth phase.

2.2. Somatostatin analogues

All somatostatin structural analogues used in this study were listed in Table 1. TT-232, TT-232 (+D-Trp), a side-product of the TT-232 synthesis, and RC-160 were synthesised. Four TT-232 derivatives were also synthesised with different amino acid sequences at the fifth position from the N-terminus. These agents were initially resolved in 0.02 N HCl with 30% dimethyl sulphoxide (DMSO) and were diluted by serum-free

Table 1
Structure of TT0232 and other somatostatin analogues

RC-160	D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2
TT-232 (+D-Trp)	D-Phe-Cys-Tyr-D-Trp-D-Trp-Lys-Cys-Thr-NH_2
TT-232	D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2
TT-232 (5-Ala)	D-Phe-Cys-Tyr-D-Trp-Ala-Cys-Thr-NH_2
TT-232 (5-Cit)	D-Phe-Cys-Tyr-D-Trp-Cit-Cys-Thr-NH_2
TT-232 (5-Arg)	D-Phe-Cys-Tyr-D-Trp-Arg-Cys-Thr-NH_2
TT-232 (5-Orn)	D-Phe-Cys-Tyr-D-Trp-Orn-Cys-Thr-NH_2

conditioned media, and stored at −30 °C. A final concentration of DMSO applied in our experiments was less than 1%; this concentration had no effect on cell growth.

2.3. *Evaluation of growth inhibition* in vitro

Cells were seeded into 12-well culture plates (3×10^4 cells/well) in 2 ml of medium and incubated for 48 h. After incubation, the cells were exposed to TT-232, TT-232 (+D-Trp), and RC-160 for 48 h at concentrations ranging from 0.1 to 100 μM. For the comparison of the four TT-232 derivatives, PANC-1 and AsPC-1 cells were exposed for 48 h to 30 μM of the agents. The medium was replaced with fresh medium immediately before exposure to the agents. The number of attached cells in monolayer was determined in triplicate with a Coulter-counter (Coulter Electronics, London, UK) to compare the antiproliferative activities among these agents.

2.4. *Cell survival fraction assay*

PANC-1 cells were exposed to TT-232 for 2 h at the concentrations of 1, 10 and 100 μM. Then, the cells were collected and centrifuged at 150*g* for 5 min. The cell pellets obtained were diluted with culture medium in order to include 500 cells per 1 ml of medium. Five hundred cells with 1 ml of culture medium were seeded into 35 mm culture dishes (Nunc, Roskilde, Denmark). Five days later, the number of colonies formed was counted in triplicate.

2.5. *Modified FITC-TUNEL staining*

PANC-1 cells exposed to 5 μM TT-232 for 48 h were subjected to fluorescent isothiocyanate-TdT-mediated dUTP-biotin nick-end labelling (FITC-TUNEL) staining to detect fragmented DNA. Details of this procedure have been previously described in Refs. [22–25]. For counter-staining of the intact nuclei, 0.5-mg/l propidium iodide in 1.12% sodium citrate was employed. The TUNEL-positive nuclei were counted on the photoslides and expressed as a percentage of the total nuclei.

2.6. *Effects of TT-232 on protein tyrosine phosphorylation*

CFPAC-1 cells were exposed to 30 μM TT-232. At 12, 24 and 36 h, the cells in attached monolayer were lysed and the supernatants collected and resuspended with the same volume of gel-loading buffer as previously described in Refs. [24,25]. For Western blotting, proteins in quantities equal to those of the lysates were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (8% gels) and transblotted onto nitrocellulose filters. Proteins were detected using anti-phosphotyrosine polyclonal antibody (4G10, upstate biotechnology, Lake Placid, USA).

2.7. *Effect of sodium orthovanadate on growth inhibition by TT-232*

Sodium orthovanadate (SO) acts as a protein tyrosine phosphatase (PTPase) inhibitor. Sodium orthovanadate (Aldrich, Steinheim am Albuch, Germany) at a final concentration ranging from 1 to 100 μM was added for 48 h to the culture medium of CFPAC-1 cells in the presence or absence of 30 μM TT-232. DMSO was added to a final concentration of less than 1% in order to facilitate the vanadate penetration. After incubation, the number of attached cells was counted as described above.

2.8. *PTPase activity assay*

CFPAC-1 cells were exposed to 30 μM TT-232, with or without 100 μM sodium orthovanadate. At 12, 24 and 36 h, the attached cells were used to assay PTPase activity. The PTPase assay was performed using Promega's non-radioactive Tyrosine Phosphatase Assay System (Promega Corporation, Madison, USA) following the manufacturer's instructions.

2.9. *Receptor binding studies*

The binding assay was conducted as described by O'Byrne and colleagues, but with some modifications [26]. Cell samples were thawed on ice and homogenised. The homogenates were then centrifuged at 500*g* for 15 min at 4 °C to remove the nuclear debris. The supernatant containing the crude membrane fraction was ultracentrifuged at 40 000*g* for 60 min at 4 °C. The final pellet was resuspended in an appropriate buffer and the protein concentration determined by the Tonein-TP kit (Ootsuka, Tokyo, Japan). The sample was then used for the receptor binding studies. ^{111}In-DTPA-TT-232 was conjugated and used as a radioligand. Binding reactions were performed for 2 h at room temperature using a competitive-inhibition method. 50 μl of membrane fraction containing 75 μg of protein was incubated with 50 μl of radioligand (corresponding to 7500 counts per minute (cpm)) in the presence or absence of unlabelled TT-232 with 50 μl of incubation buffer, which was composed of 50 mM Tris base, 5 mM $MgCl_2$, 0.25 mM phenylmethylsulphonylfluoride (PMSF), 20 mg/l bacitracin and 0.2% bovine serum albumin (BSA). The reaction was terminated by rapid filtration through glass fibre filters (Whatman GF/B). The radioactivity of the filters was counted in a gamma-counter. To determine the types of receptor binding, the dissociation

constant (K_d), and the maximal binding capacity of receptors (B_{max}) were calculated. The binding data were analysed by the Scatchard method.

2.10. *Evaluation of growth inhibition* in vivo

AsPC-1 cells (1.5×10^6) were subcutaneously (s.c.) inoculated into athymic male nude mice (Balb/c nu/nu, SRL, Japan) that were bred and maintained in pathogen-free conditions. These mice were used as transplant recipients at the age of 6 weeks, and were divided into three groups. The first group of mice received TT-232 administration twice a day intraperitoneally (i.p.) at the dose of 5 mg/body weight (kg) right after the cell inoculation, and the second group received TT-232 after the tumour formation. The nude mice of the third group were employed as controls. Tumour volume was determined by measuring in two dimensions with vernier calipers and by using the equation of $V=W^2\times L/2$, in which $W=$width and $L=$length of tumour. The comparative statistical evaluation among groups was performed by a two-way analysis of variance (ANOVA). A probability level of <0.05 was considered to be significant.

3. Results

3.1. *Growth inhibition* in vitro

Growth inhibition curves of human pancreatic cancer cell lines after exposure to TT-232 for 48 h were shown in Fig. 1. TT-232 inhibited the growth of all cell lines used in this experiment in a dose-dependent manner. The concentrations required for 50% growth inhibition (IC_{50}) were approximately 5, 7, 10, 10 and 15 μM in PANC-1, MIAPaCa-2, CAPAN-2, AsPC-1 and CFPAC-1 cells, respectively. RC-160 had no growth inhibitory effect on all of the cell lines at micro-molar concentrations (Fig. 2). RC-160 had a less than 10% growth inhibitory effect on MIAPaCa-2 cells only at nano-molar concentrations (data not shown) and an approximately 30% proliferative effect at micro-molar concentrations. TT-232 (+D-Trp) had no effect on the growth of all of the cell lines (Fig. 2). 1–100 μM of TT-232 did not affect the cell survival fraction (Table 2), indicating that the antiproliferative activity of TT-232 was not a non-specific effect with the concentrations used in this experiment. As for the TT-232 derivatives, they showed differences in the antiproliferative activities on PANC-1 cells and AsPC-1 cells (Fig. 3). The relative strength of activities were in the order of TT-232 (5-Orn), TT-232 (5-Arg), TT-232 (5-Cit) and TT-232 (5-Ala).

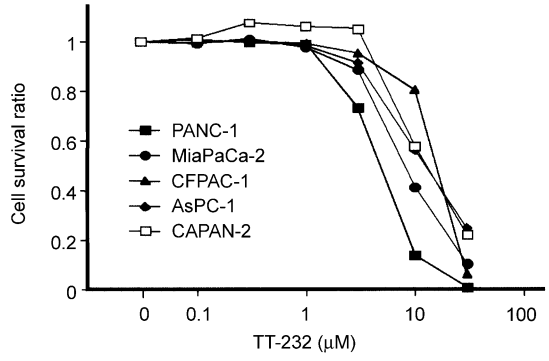

Fig. 1. Growth inhibitory effect of TT-232 on human pancreatic cancer cell lines. Cells were exposed to TT-232 for 48 h in their exponential growth phase. The number of cells in the attached monolayer was counted in triplicate and the mean plotted.

3.2. *TUNEL staining*

PANC-1 cells were exposed to TT-232 for 48 h at the concentration of 5-μM, which was equivalent to the IC_{50} of the cells for growth inhibition. Affected cells were detached from the monolayer and TUNEL-positive nuclei of the detached cells were observed as light-green signals in the fluorescence microscope as previously described in Refs. [24,25]. The TUNEL-positive ratio of the treated cells was approximately $25\pm5\%$ and that of non-treated (control) cells was less than 5% (Fig. 4).

3.3. *Effect of TT-232 on protein tyrosine phosphorylation*

The effects of 30 μM TT-232 on the level of protein tyrosine phosphorylation were examined by an immunoblot analysis in CFPAC-1 cells. TT-232 caused a selective dephosphorylation of the corresponding protein bands, approximately 170 and 120 kDa, that were highly phosphorylated in the control cells at 12 and 24 h. Up to 36 h, TT-232 treatment continued to result in dephosphorylation of the 170-kDa protein band. In contrast, TT-232 caused a selective phosphorylation of an approximately 75-kDa protein band that was less phosphorylated in the control cells (Fig. 5a).

3.4. *Effect of sodium orthovanadate on cell growth inhibition by TT-232*

The growth inhibitory effect of 30 μM TT-232 on CFPAC-1 cells was antagonised by SO in a dose-dependent fashion (Fig. 5b). More than 90% inhibition of cell growth was observed by addition of 30 μM TT-232 alone, but only 10% inhibition was detected by addition of 30 μM TT-232 plus 100 μM SO. 100 μM SO alone did not influence the cell growth.

3.5. *PTPase assay*

The PTPase activity was significantly stimulated by the administration of 30 μM TT-232 and showed a

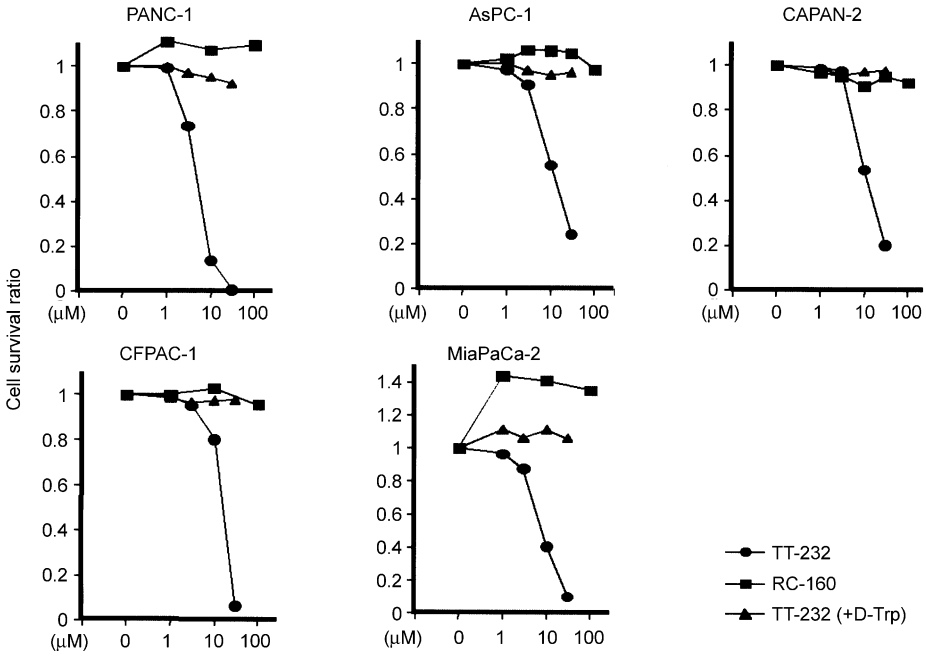

Fig. 2. Growth inhibitory effects of TT-232, and comparison with RC-160 and TT-232 (+D-Trp). The number of cells in the attached monolayer was counted in triplicate and the mean plotted.

Table 2
Cell survival fraction assay

	The number of colonies Mean±S.E.M.
Control	211±13.9
TT-232	
1 μM	194±3.7
10 μM	218±11.9
100 μM	217±25.5

S.E.M., standard error of the mean.

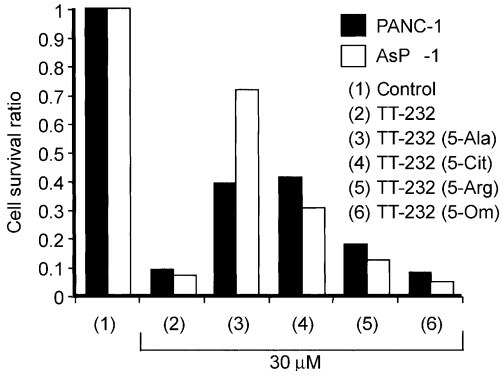

Fig. 3. Structure–activity relationship studies of various TT-232 structural analogues. The number of cells in the attached monolayer was counted in triplicate and the means expressed in a bar graph.

maximum activity after 24 h of incubation. At this time point, TT-232 increased the PTPase activity more than 3-fold over the control level as shown in Fig. 5c. In contrast, the administration of TT-232 with SO did not result in any significant change in PTPase activity.

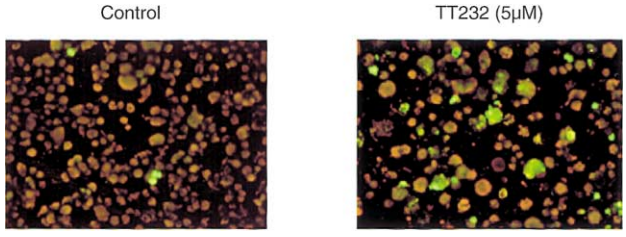

Fig. 4. Apoptotic cell death induced by TT-232. Approximately 25% of the detached cells were stained positively (green) in the TT-232 treated cells, but <5% were stained in the control cells (FITC-TUNEL method; original magnification 100×).

3.6. *Receptor binding*

Among the five human pancreatic cancer cell lines evaluated for the presence of binding for ^{111}In-TT-232, a significant difference between total and non-specific binding was observed in one cell line, MIAPaCa-2; however, specific binding of TT-232 was not detected in other cell lines. The specific binding site for TT-232 on the surface of MIAPaCa-2 cells was a low affinity site ($K_d = 1.59 \times 10^{-8}$) and of low capacity ($B_{max} = 4.62$ fmol/mg membrane protein).

3.7. *Growth inhibition* in vivo

The effects of TT-232 administration on the tumour growth of a xenograft model are shown in Fig. 6. TT-232, when administered simultaneously with the inoculation of cancer cells, significantly inhibited the tumour growth reaching an 80% inhibition by the 32nd day

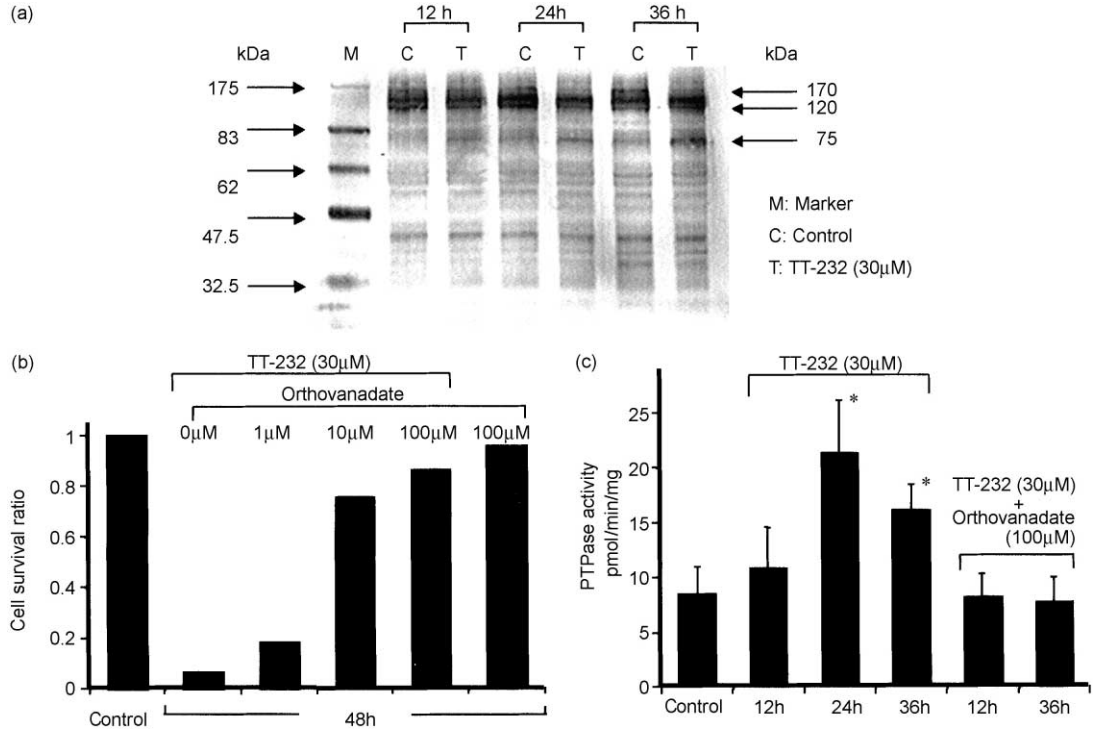

Fig. 5. Protein tyrosine phosphatase activities related to TT-232. (a) Levels of protein tyrosine phosphorylation. Anti-phosphotyrosine immunoblot of CFPAC-1 cells lysates (antibody: 4G10). (b) Inhibition of the antiproliferative activity of TT-232 by sodium orthovanadate, a tyrosine phosphatase inhibitor. The number of cells in the attached monolayer was counted in triplicate and the mean expressed in a bar graph. (c) Effect of TT-232 on protein tyrosine phosphatase activity. Experiments were performed in triplicate and repeated three times. *Significant increase against control ($P<0.05$, Students' t-test).

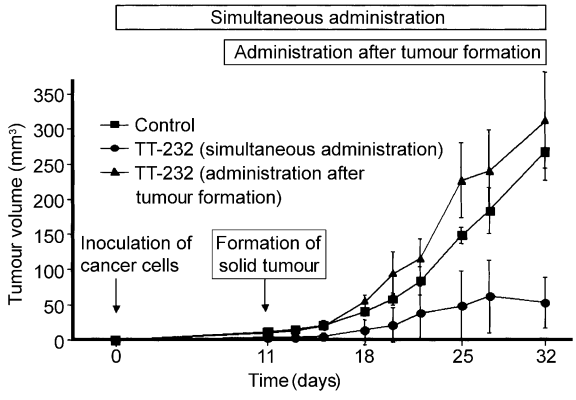

Fig. 6. Effect of TT-232 on xenograft formation by AsPC-1 cells. Exponentially growing cells were inoculated subcutaneously (s.c.) (1.5×10^6 cells/site) into athymic nude mice. TT-232 was injected intraperitoneally at the dose of 5 mg/kg of body weight twice a day. Values are means ± standard error of the means (S.E.M.) ($n=3$).

relative to the controls. TT-232, when administered immediately after the tumour formation, did not significantly affect the tumour growth.

4. Discussion

In this study, the antiproliferative activity of TT-232, a novel somatostatin analogue, was examined in human pancreatic cancer cells. The growth of PANC-1, AsPC-1, CAPAN-2, CFPAC-1, and MIAPaCa-2 cells was inhibited by TT-232, but not by RC-160 nor by TT-232 (+D-Trp) (Figs. 1 and 2). RC-160 and TT-232 (+D-Trp) have a usual six-residue ring structure, while TT-232 has a unique five-residue ring structure. RC-160 has a strong affinity to somatostatin receptor subtype 2 (SSTR2) and mediates the antiproliferative activities most effectively among the somatostatin receptor subtypes (SSTR1–5) [27,28]. The SSTR2 was a major type of receptor among somatostatin receptor subtypes in normal pancreas, whereas it is less often observed in pancreatic cancer [29]. Among the cell lines used in this study, only MIAPaCa-2 cells expressed SSTR2 [10,17,29] and SSTR4 [7]. These facts may explain why RC-160 did not influence the growth of the cell lines, but inhibited the growth of MIAPaCa-2 cells at nanomolar concentrations (data not shown). However, we did demonstrate that TT-232 showed antiproliferative activity in all of human pancreatic cancer cell lines used in this study. The antiproliferative activity of TT-232 was not due to a non-specific cell toxicity, but likely due to some kind of specific action that was correlated with the replacement of the fifth amino acid from the N-terminus (Fig. 3). Furthermore, TT-232 induced apoptotic cell death and the TUNEL-positive ratio reached up to approximately 25% in PANC-1 cells at the dose of 5

μM TT-232, which corresponded to the dose of IC_{50} for the growth (Fig. 4).

In general, the antiproliferative activities of somatostatin and somatostatin analogues have been reported to be closely related to the activation of protein tyrosine phosphatase (PTPase) [30,31], partially related to the inhibition of cyclic adenosine monophosphate (cAMP) formation [32] and intracellular effectors, such as Na^+/K^+-adenosine triphosphate (ATPase) [33]. We examined the relationship between PTPase activities and growth inhibitory effects of TT-232 on pancreatic cancer cells. Administration of TT-232 at a concentration of 30 μM changed the levels of protein tyrosine phosphorylation in human pancreatic cancer cells. Tyrosine phosphorylations of proteins at approximately 170 and 120 kDa decreased after 12 h of incubation with TT-232 and decreased even more after 24 h of incubation (Fig. 5a). This phenomenon may provide a plausible explanation for the growth inhibitory effect of TT-232, which was observed after 48 h of incubation. The decreased level of protein tyrosine phosphorylation caused by TT-232-induced PTPase activation was closely related to its antiproliferative activity. The antiproliferative activity was probably the result of the inhibition of tyrosine phosphorylation. In fact, sodium orthovanadate, which acts as a PTPase inhibitor, significantly blocked the antiproliferative activity of TT-232 up to 90% compared with the controls (Fig. 5b). Although vanadate did not completely abolish the antiproliferative activity of TT-232, this is probably due to the inhibitory effect on cAMP formation that is not blocked by vanadate [32,34]. Direct measurements of TT-232 stimulation of PTPase activity strongly support the major and essential involvement of PTPase in the inhibition of the growth induced by TT-232 (Fig. 5c).

The stimulation of the PTPase activities by TT-232 was sustained at least up to 24 h in the case of the PTPase responsible for dephosphorylation of the 120-kDa protein, and at least up to 36 h in PTPase(s) responsible for the dephosphorylation of the 170-kDa proteins (Fig. 5a). Vantus and colleagues reported that TT-232 induced a biphasic activation of PTPase in the human colon tumour cell line, SW620, in a PTPase assay [35]. In our study, a 75-kDa protein was highly phosphorylated by TT-232; however, this activity had no influence on the *in vitro* growth inhibitory effect of TT-232. With regard to somatostatin, G protein-coupled receptors were reported to conduct growth inhibitory signals mediated by PTPase stimulation [11], but the effector molecules that act downstream of G protein-coupled receptors are not known. The relationship between somatostatin receptor subtypes and their capacity for stimulation of PTPase activity are not clearly known; most investigators are in agreement with respect to the capacity for SSTR2 and SSTR4 to stimulate PTPase activity, but there are still conflicting views with regard to data for SSTR1, SSTR3 and SSTR5 [11,12,36–42]. Moreover, a Chinese hamster ovary (CHO) cell line, which did not express SSTR2, failed to increase PTPase activity after transfection with rat *sstr2*, but did show increased PTPase activity after transfection with a mouse or human *sstr2* [12,16,42]. To summarise, it has not yet been elucidated how somatostatin exerts its antiproliferative activity through PTPase stimulation. The pattern of PTPase modification by TT-232, which was shown in this study, suggests that the mechanism of the antiproliferative activities of somatostatin and its analogues should be further investigated.

In the receptor binding assay, the specific binding of TT-232 was demonstrated in MIAPaCa-2 cells. In nine human pancreatic carcinoma cell lines (AsPC-1, BxPC-3, CAPAN-1, CAPAN-2, HS766T, MIAPaCa-2, PANC-1, SU.86.86, and CAV), Fisher and colleagues examined the expression of messenger RNA (mRNA) of the somatostatin receptor subtypes 1–5 by use of reverse transcription and the polymerase chain reaction, and examined the translation of mRNA into functional cell surface receptors by a classic competitive binding assay. They reported that only MIAPaCa-2 cells had functional cell surface somatostatin receptors, despite the prevalence of receptor gene expression, and that the genes for SSTR1, SSTR2 and SSTR5 were transcribed in the majority of the cultured cell lines [17]. For explanations of this discrepancy, they suggested that the number of cell surface receptor might be too low to be detected, or that there was a post-transcriptional defect in the tumour cells. Moreover, *sstr2* was reported to be absent in most pancreatic carcinomas of human origin and their metastasis. Taking these facts together, our findings raise the possibility that TT-232 binds weakly, but specifically, with a certain SSTR other than SSTR2, or binds to a still unknown receptor that are more abundant in MIAPaCa-2 cells than the other cell lines. Furthermore, to our knowledge, this study is the first to demonstrate that the effect of TT-232 is mediated through a specific receptor.

In this study, we also examined the *in vivo* growth inhibitory effect of TT-232 in order to gain an insight in its use as a therapeutic agent. TT-232, given twice-a-day, with the tumour cell inoculation was effective in delaying tumour formation, but had no effect on tumour growth when administered after tumour formation (Fig. 6). The injected dose of 5-mg/kg i.p. roughly corresponded to more than 30 μM in the nude mouse. This dose of TT-232 may not be enough to block the factors necessary to form a tumour from inoculated cells. The failure to inhibit tumour growth is likely due to unknown factors, such as the function of metabolites of TT-232 *in vivo*, the effect of various neurohormones that affect PTPase activity, and the significance of PTPase inhibitory effect of TT-232. Another explana-

tion may be that an i.p.. twice-a-day administration was insufficient to maintain the optimal concentration of TT-232 in the blood compared with a constant infusion of the analogue.

In conclusion, TT-232 inhibited the growth of human pancreatic cancer cell lines in a dose-dependent manner probably through a specific-receptor mediated mechanism. Apoptosis was found to be involved in the growth inhibition, which was closely related to PTPase activation. TT-232 delayed the growth of the tumour *in vivo*. Although it is often difficult to translate such experimental efficacy (as shown in our data) into the therapeutic clinical applicability of TT-232 our study has laid the groundwork for the development of TT-232 as a possible therapeutic modality in pancreatic cancer.

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