Original Paper

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The Tumor-Selective Somatostatin Analog, TT2-32 Induces a Biphasic Activation of Phosphotyrosine Phosphatase Activity in Human Colon Tumor Cell Line, SW620

Abstract

Somatostatin has been demonstrated to activate phosphotyrosine phosphatases (PTPases) in pancreatic cells. In this work we studied the effect of a tumor-selective somatostatin structural derivative, TT2-32, on the PTPase activity in the SW620 human colon tumor cell line. TT2-32 caused a strong inhibition of cell proliferation. In response to TT2-32 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 TT2-32. TT2-32-induced activation of PTPases may be an important early step in the signaling cascade in the inhibition of cell proliferation in colon carcinomas.

Key Words

Phosphotyrosine phosphatase
Somatostatin
Colon tumor

Introduction

The cyclic tetradecapeptide somatostatin (S14) [1] has pleiotropic biological functions as a regulator of different secretion processes, and as a neurotransmitter. In addition, S14 shows an antiproliferative effect both in vitro and in vivo and can be considered as an endogenous antiproliferative agent [2]. Somatostatin can regulate multiple cellular effector systems, including adenyl cyclase [3], K^+ channels [4], voltage-dependent Ca^{2+} channels [5] and protein phosphatases [6]. Recent studies have shown that somatostatin can induce phosphotyrosine phosphatase (PTPase) activation in pancreas, in rat pancreatic acinar cells [7] and in the human tumor pancreatic cell line (MIA PaCa-2) [6].

We have developed a novel selective somatostatin structural derivative [8, 9, 15] which has strong antitumor activity both in vitro and in vivo without inhibiting growth hormone release. We have also demonstrated that the antiproliferative effect of TT2-32 correlates with a significant inhibition of tyrosine kinase activity following a 24-hour incubation [9].

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In the present study we investigated the effect of our novel somatostatin structural derivative, TT2-32, on cell proliferation, on PTPase activation and on the activity of tyrosine kinases, investigating the effect of this structural derivative on the short-term signalling pathway in the human colon tumor cell line, SW620. We demonstrated that TT2-32 strongly inhibits cell proliferation in these cells. We could measure a biphasic transient PTPase activation but we could not find a significant effect on tyrosine kinase activity at the same time. Our results suggest that the activation of PTPase might be involved in the signal transduction pathway through which this tumor-selective somatostatin analog can inhibit cell proliferation.

Materials and Methods

Materials

Somatostatin (S14) was obtained from Bachem (Bubendorf, Switzerland), Sandostatin (SMS) was kindly provided by Sandoz (Basel, Switzerland), TT2-32 [*D*-Phe-Cys-Tyr-*D*-Trp-Lys-Cys-Thr(NH_2)] was developed and synthesized in our laboratory as described earlier [9]. $E_{11}G_1$ (Glu-Asp-Ala-Glu-Tyr-Ala_2-Arg_3-Gly) was prepared in our laboratory by solid phase peptide synthesis. The SW620 (CCL 227) human colon tumor cell line was obtained from the American Type Culture Collection.

Culture of SW620 Cells

SW620 cells were cultured in Leibovitz's L-15 medium supplemented with 10% fetal calf serum (FCS) in a humified atmosphere of 95% air and 5% CO_2 at 37°C. In cell growth studies with somatostatin, 350,000 cells were seeded per well in a culture medium of 2 ml in 6-well plates (Nunclone). Cells were incubated for various times with the indicated test substances in the absence of FCS. For cell growth assays after trypsinization and dispersion cells were dyed with trypan bue and counted in a hemocytometer.

Preparation of SW620 Cell Homogenate

Tumor cells were homogenized using a Dounce homogenizer in homogenization buffer (50 m*M* Tris-HCl, pH 7.8, 50 m*M* $MgCl_2$, 100 μ*M* Na_3VO_4, 1 m*M* EDTA, 50 μg/ml aprotinin, 1 m*M* phenylmethane sulfonylfluoride). Protein concentration of SW620 cell homogenate was determined using the protein determination methods of Lowry et al. [10] or Bradford [11] with bovine serum albumin as standard.

Assay for PTPase Activity

PTPase activity was measured by the release of [^{32}P]-orthophosphate from ^{32}P-labeled synthetic peptide, corresponding to the autophosphorylation region of the insulin receptor as described earlier [12]. The insulin receptor preparation used for the phosphorylation of the synthetic peptide was isolated from human placenta using wheat germ agglutinin affinity chromatography. The 50-μl reaction mixture contained 25,000 cpm of ^{32}P-labeled synthetic peptide, 50 m*M* HEPES with 0.1 w/v% Triton X-100, pH 7.4, 1 m*M* dithiothreitole, 2 m*M* EDTA, and 20 μl cell extract (40–60 μg of protein). The reaction was allowed to proceed for 5 min at 30°C, then stopped by the addition of 5 m*M* silicotungstate/1 m*M* H_2SO_4. The liberated inorganic [^{32}P]phosphate was extracted using the molybdate extraction procedure and radioactivity was evaluated by liquid scintillation. The amount of $^{32}P_i$ release was determined from the specific radioactivity of [γ-^{32}P]ATP used for the phosphorylation reaction.

One unit of PTPase activity was defined as the amount which released 1 pmol phosphate/min at 30°C from radiolabeled substrate.

Assay for Tyrosine Kinase Activity

Tyrosine kinase activity was measured according to the method of Swarup et al. [13]. The reaction volume of 240 μl contained 50 m*M* Tris-HCl, pH 7.8, 50 m*M* $MgCl_2$, 100 μ*M* sodium *o*-vanadate, 0.1% Nonidet P-40, 24 μ*M* ATP, 0.5 nmol [γ-^{32}P]ATP, 1 m*M* of the substrate $E_{11}G_1$, and 60 μl of cell homogenate (40–60 μg of protein). The assay was initiated by the addition of [γ-^{32}P]ATP. After incubation for 10 min at 30°C the reaction was stopped by the addition of 150 μl 10% trichloroacetic acid, and 10 μl 20 mg/ml BSA. The precipitated protein was removed by centrifugation (3,200 *g*, 25 min) and two 70-μl aliquots of the supernatant were spotted on phosphocellulose paper (Whatman P81). Phosphocellulose paper squares were washed 5 times in 0.5% phosphoric acid and once in acetone. The dried papers were counted for radioactivity in 5 ml of scintillation fluid. For each sample, an appropriate reaction mixture without the peptide substrate was run as control.

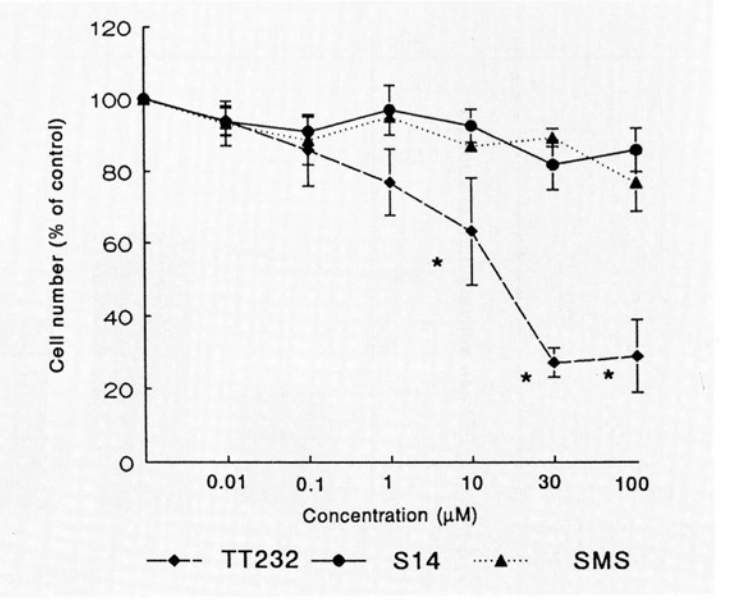

Fig. 1. Effects of somatostatin analogs on growth of SW620 cells after 24 h of treatment. SW620 cells were cultured and cell growth assays were performed as described in Materials and Methods. Values are expressed as a percentage of the number of control (untreated) cells ($1.8 \cdot 10^6 \pm 94 \cdot 10^3$ cells) and are the means ± SEM of three separate experiments. * $p < 0.001$ (n = 3), using the Student's t test.

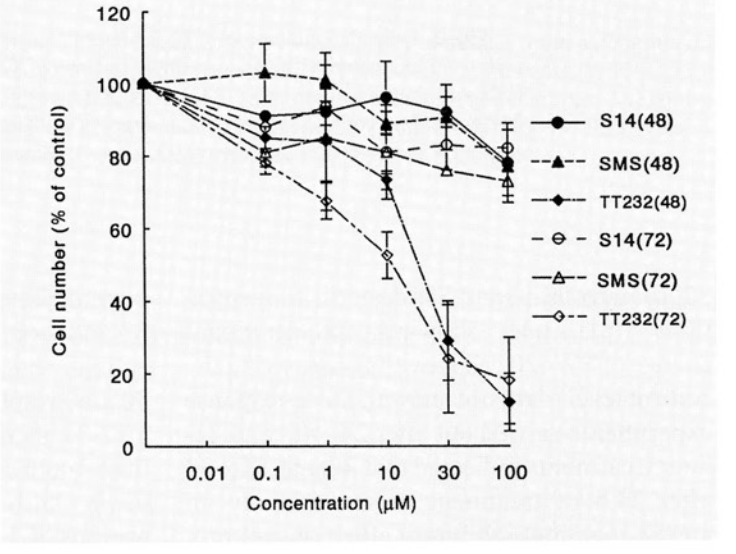

Fig. 2. Effects of somatostatin analogs on growth of SW620 cells after 48 and 72 h of treatment. SW620 cells were cultured and cell growth assays were performed as described in Materials and Methods. Values are expressed as a percentage of the number of control (untreated) cells and are the means ± SEM of three separate experiments.

Results

Effect of Somatostatin on SW620 Cell Growth

The effect of somatostatins was tested in the exponentially growing SW620 human colon tumor cell line. The somatostatin structural derivative TT2-32 at 10, 30 and 100 μ*M* final concentrations exerted a significant inhibition on SW620 cell growth after 24, 48 and 72 h of treatment. In contrast, somatostatin and Sandostatin had no significant effect on cell growth in this system (fig. 1, 2). Experiments were also carried out using lower TT2-

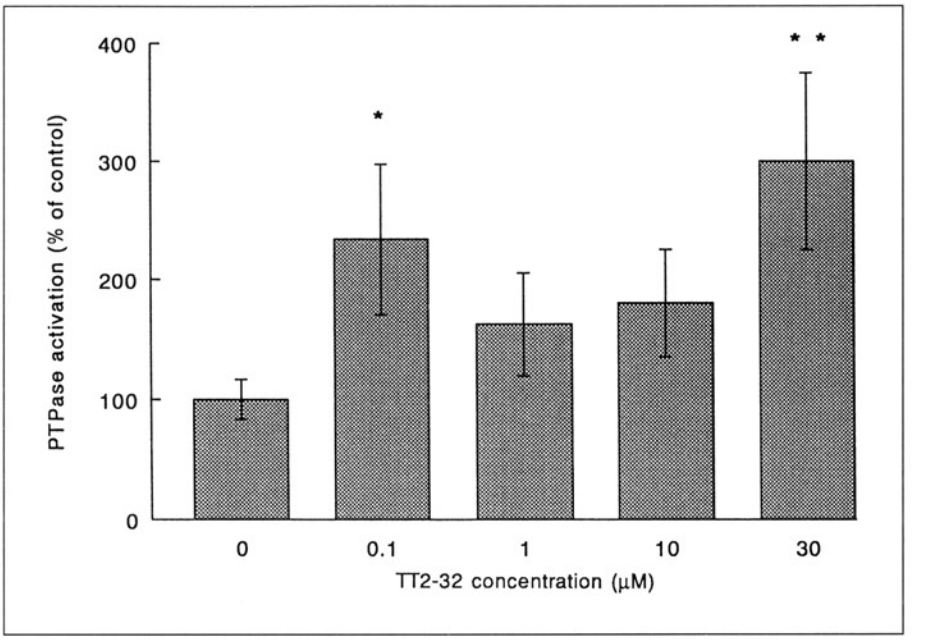

Fig. 3. Dose dependence of TT2-32-stiumlated PTPase activity in SW620 cells. SW620 cells were incubated with various concentrations of TT2-32 for 10 min at 37 °C. Extraction of cells and the measurement of PTPase activity was performed as described in Materials and Methods. The control (100%) value denotes a PTPase activity of 2.26 pmol ^{32}P/min · mg protein. * $p < 0.05$ (n = 3); ** $p < 0.025$ (n = 4), using the Student's t test.

32, somatostatin, and Sandostatin concentrations (0.1–1 n*M*) showing no significant change of SW620 cell growth compared to the control level [data not shown]. Dose-response experiments carried out after 24-, 48- and 72-hour treatments indicated that 30 μ*M* TT2-32 after 24 h of treatment exerted already an almost maximal inhibitory effect on cell proliferation with more than 70% inhibition compared to the control value.

Characterization of PTPase Activity of SW620 Cells

Treatment of SW620 cells with the somatostatin structural derivative TT2-32 resulted in a significant increase of PTPase activity in a time- and dose-dependent manner. Our novel somatostatin structural derivative TT2-32 induced a biphasic increase of PTPase activity showing two maxima at 100 n*M* and 30 μ*M*, respectively. At these concentrations TT2-32 increased the PTPase activity more than 2-fold over baseline level as shown in figure 3. On the contrary, intermediate concentrations of TT2-32 (1 and 10 μ*M*, respectively) did not cause any significant change in PTPase activity. A time course of the effect of 100 n*M* and 30 μ*M* TT2-32 on PTPase activity is shown in figure 4. SW620 cells responded to TT2-32 with a rapid and sustained (from 5 to 30 min) increase of PTPase activity. The maximal effect was 3 times more than the control level after addition of 30 μ*M* TT2-32 at an incubation time of 10 min. Our mea-

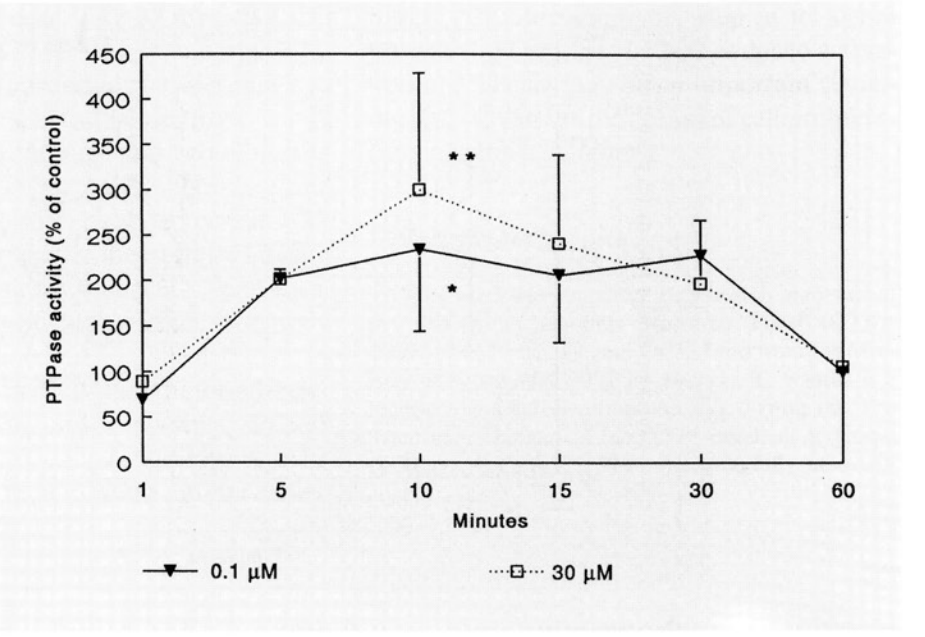

Fig. 4. Time course of TT2-32-stimulated PTPase activity in human colon carcinoma cell line. SW620 cells were incubated with 0.1 and 30 μ*M* TT2-32 for various times at 37 °C, were extracted and assayed for PTPase activity as described in Materials and Methods. The control (100%) value denotes a PTPase activity of 2.26 pmol ^{32}P/min · mg protein. Each value is representative of three independent experiments. * $p < 0.05$; ** $p < 0.025$.

surements show that TT2-32 produces a strong increase of PTPase activity in SW620 cells in a biphasic manner, lasting from 5 to 30 min.

Tyrosine Kinase Activity Measurements

After treatment of SW620 colon tumor cells with the somatostatin structural derivative TT2-32 we also determined the tyrosine kinase activity detecting no discernible change of the baseline activity. The only effect on tyrosine kinase activity was an approximately 30% inhibition, after treatment with 0.1 and 30 μ*M* TT2-32 at an incubation time of 30 min as is shown in figure 5. Tyrosine kinase was also determined after treatment with intermediate (1 and 10 μ*M*) TT2-32 concentrations for various times showing only a minor inhibition similar to that at 0.1 and 30 μ*M* [data not shown].

Discussion

Somatostatin exerts multiple cellular actions throughout the body. Among others, several reports have demonstrated that somatostatin can inhibit cell proliferation in vivo and in vitro [2]. However, the signal transduction pathway of somatostatin has not yet been elucidated. PTPase are likely candidates in this process, because activation of PTPase might reverse protein tyrosine kinase activity which can be induced by a growth factor-

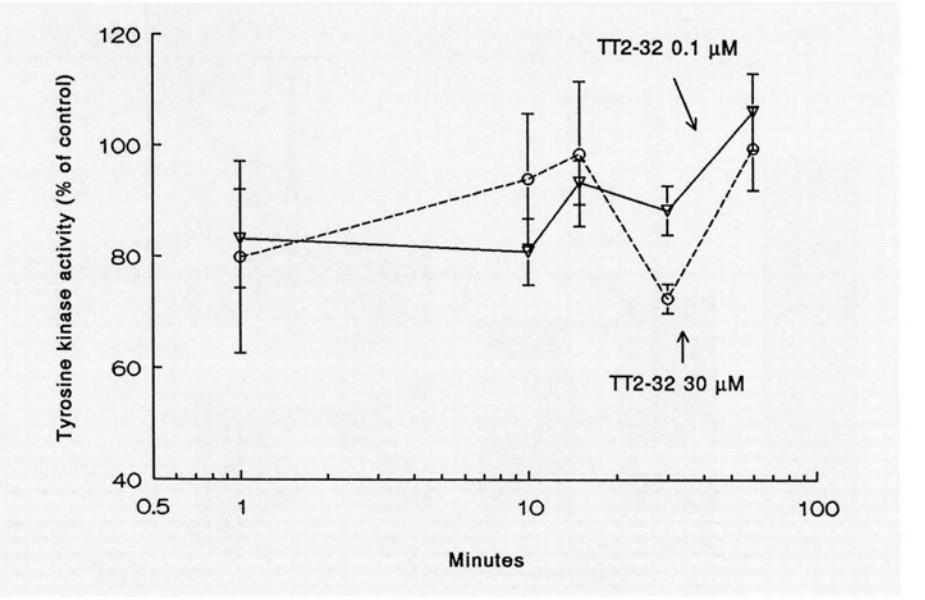

Fig. 5. Time course of the effect of TT2-32 on tyrosine kinase activity in SW620 cells. Cells were treated with 0.1 and 30 μ*M* TT2-32 for various times at 37°C. Cell extracts were prepared and tyrosine kinase assays were run as described in Materials and Methods. Each value is representative of at least three separate experiments. The control (100%) value denotes a tyrosine kinase activity of 0.137 pmol ^{32}P/min · mg protein.

induced mitogenic signal. Indeed, recent papers proved that somatostatin could activate PTPase in pancreatic cells [6, 7].

We have developed a tumor-selective somatostatin analog TT2-32 which has a strong antiproliferative effect both in vitro and in vivo without inhibiting growth hormone release [9, 14]. In this study, we analyzed the effect of this somatostatin analog on signal transduction in a human colon tumor cell line SW620. This novel somatostatin structural derivative induced a strong inhibition of cell proliferation in these cells, where native somatostatin and Sandostatin were ineffective. We have previously demonstrated that TT2-32 is a potent inhibitor of cell growth in various human breast, prostate and colon tumor cell lines [8, 9] while Sandostatin was found to be ineffective in modifying the proliferation of intestinal tumors (including colon) and crypt cells in azoxymethane-treated rats [15].

To get a better insight into the mechanism of action of this somatostatin analog in SW620 colon tumor cells we characterized the activity of PTPases after addition of TT2-32. We measured a biphasic PTPase activation in a time- and dose-dependent manner. The stimulatory effect reached a significant increase after 5 min of incubation and remained high for 30 min, data which correlated well with those obtained in pancreatic acinar cells [7]. TT2-32 has a unique mechanism of action inducing a double maximal increase of PTPase activity in these colon tumor cells. The measured biphasic fashion of the dose-response curve for TT2-32 suggests the possible existence of more than one receptor population with different ligand binding affinity

states on SW620 cells. TT2-32 may also activate different PTPases at low and high concentrations. There are ongoing experiments to test this hypothesis in our laboratory.

In contrast to the fact that somatostatin-induced PTPases can dephosphorylate and inactivate membrane receptor tyrosine kinases [6], we failed to measure significant tyrosine kinase inhibition in short-term incubation which may be due to the relative insensitivity of the assay used.

Our data show that the tumor-selective somatostatin structural derivative TT2-32 strongly stimulates PTPase activity in a biphasic manner in a SW620 human colon tumor cell line as an early step in its signal transduction process. TT2-32-induced activation of PTPases may be an important signaling pathway in the inhibition of cell proliferation in colon carcinomas.

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