Abstract. Background: It was previously demonstrated that octreotide (Sandostatin) induced an increased apoptotic activity in human pancreatic cancer xenografts after a high dose, 1-month treatment. In the present study the effect of smaller doses (2x100 μg/kg b.w.) administered in a short-term (4-day) experiment were investigated. Materials and Methods: CBA immunosuppressed mice bearing human pancreatic carcinoma (PXZ-40/6) were treated daily with 2x100 μg/kg b.w. Sandostatin subcutaneously for 4 consecutive days. The number of tumor cells displaying apoptotic bodies (late event) and mitotic activity were assessed by morphometry, while the earlier phase of the apoptotic process was determined using flow cytometry. Results: A short octreotide treatment did not influence the mitotic activity, but the number of apoptotic cells decreased significantly (1.8±0.44/mm² in controls vs. 6.8±1.0/mm² in treated tumors, p<0.0009). The percentage of nuclei in sub-G1 phase almost doubled (6.0±0.75% in controls, 11.2±0.97% in the octreotide-treated group, p<0.0014). The DNA index and the proliferation indices proved to be unchanged. Conclusion: The results suggest that low doses of octreotide induce apoptosis in human pancreatic cancer xenografts after a short-term treatment.

Somatostatin analogs (octreotide, lanreotide, vapreotide) are classically used for the treatment of acromegaly, carcinoid syndrome or gastro-entero-pancreatic neuroendocrine tumors. Their tumor-inhibiting effects are governed by complex direct and indirect mechanisms, including somatostatin receptor (SSTR)-mediated effects, antagonizing growth factor and hormone-release, regulating the immune system, triggering apoptosis or inhibition of tumor angiogenesis (1). These multiple actions explain why the SSTR-negative, non-endocrine tumors may also respond to somatostatin therapy.

Octreotide is known to induce apoptosis in activated lymphocytes (2), but an increased apoptotic activity in various malignant neoplasms has also been reported in different in vitro and in vivo experimental systems. In hepatocellular carcinoma cells, apoptosis was significantly elevated in a dose-dependent manner (3) and in human gallbladder cancer cells flow cytometric studies also revealed an increased sub-G1 peak accompanied by morphological signs of chromatin condensation or formation of apoptotic bodies (4). Similar results were obtained in xenografts: octreotide or lanreotide treatments of Colo-38 carcinoma resulted in an increased apoptotic index that was compatible with that of 5-FU administration, but the combined treatments yielded no additive effect (5). Interestingly enough, however, in patients with GH-secreting pituitary adenomas there was no significant difference between the apoptotic index in the analog-treated and untreated cases (6).

After promising preclinical studies, it was reported that human pancreatic cancers were also sensitive to hormonal manipulations: combination treatments with tamoxifen and octreotide resulted in a prolonged median survival and the quality of life was significantly improved (7, 8). Using pancreatic cancer xenografts, the somatostatin receptor 2 gene transfer led to an elevated apoptotic index and the tumor sizes were reduced (9). In our previous studies, flow cytometric and TUNEL-based immunohistochemical evidence indicated that after a high-dose administration (500 μg/kg b.w. twice a day for 4 weeks) Sandostatin induced significant apoptosis in subcutaneously growing human pancreatic carcinomas in mice, accompanied by a significantly reduced cytoplasmic phosphorylation state (10, 11). Since these results reflect the end-point of a 1-month-experiment and the earlier events are unknown, the effect of octreotide with smaller doses was investigated in a short-time experiment on a xenograft system.

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Materials and Methods

Inbred CBA mice (± 20 g) were artificially immunosuppressed by thymectomy, followed by a whole-body irradiation and bone marrow reconstruction from syngenic animals as described previously (12). Human pancreatic cancer xenografts (PZX-40/6) were transplanted subcutaneously into the back region and when the tumors reached the diameter of 0.4-0.5 cm, the mice were randomized. One group (n=11) was treated daily with 2x100 µg/kg b.w. Sandostatin subcutaneously for 4 consecutive days (0.1 ml per animal), while the control mice (n=7) were given saline only. Animals were sacrificed 5 hours after the last octreotide dose. The tumors were histologically evaluated in formalin-fixed, paraplast-embedded materials. The number of mitotic figures and tumor cells containing apoptotic bodies of non-necrotic areas were counted in 30 high power microscopic fields (HPF) with an area of 0.196 mm², their final number was extrapolated for 1 mm² and the results were expressed as mean±S.E. For assessing statistical significance, the Student’s t-test was used.

For flow cytometric evaluation, fresh tumor samples were used according to the method originally described by Nicoletti et al. (13). The central necrotic parts were discarded and 5-10 mm³ of viable tumor tissues were homogenized in 0.9% saline. After resuspending the pelleted cells, the samples were incubated overnight at 4°C in a hypotonic staining solution consisting of 0.1% sodium citrate, 0.1% Triton X-100 supplemented with 100 µg/ml RNase and 50 µg propidium iodide. Nuclei stained by propidium iodide were then analyzed by a flow cytometer and the percentage of cells in sub-G1 region was estimated by Consort 30 software (Becton Dickinson, San Jose, CA, USA).

Results

The PZX-40/6 human pancreatic cancer was a well-differentiated adenocarcinoma with moderate amount of acian-blue positive mucin production and the degree of differentiation was not shifted. No measurable volume changes were seen in any xenografted tumor. All the neoplasms were aneuploid with a DNA-index of 1.6. The treatment did not alter the number of mitotic figures in the tumorous elements (3.6±0.32/mm² in controls vs. 4.3±0.7/mm² in Sandostatin-group, N.S.), however, the apoptotic activity was significantly increased (1.8±0.44 apoptotic cells/mm² in controls vs. 6.8±1.0/mm² in treated tumors, p<0.0009) accounting for more than a 3-fold elevation. Similar changes were not observed in the non-parenchymal elements (e.g., in the stroma). The presence of apoptotic bodies represents a late event of programmed cell death, but the earlier phases were also well characterized by flow cytometric studies. The data in Table I show that the low percentage of sub-G1 cells in the control samples (6.0±0.75%) almost doubled (11.2±0.97%) in the octreotide-treated group. These differences were also statistically significant (p<0.0014). No changes were noted regarding the apoptosis index in the stroma). The presence of apoptotic bodies represents a late event of programmed cell death, but the earlier phases were also well characterized by flow cytometric studies. The data in Table I show that the low percentage of sub-G1 cells in the control samples (6.0±0.75%) almost doubled (11.2±0.97%) in the octreotide-treated group. These differences were also statistically significant (p<0.0014). No changes were noted regarding the DNA-index between the two groups (1.58 in controls, 1.59 in treated tumors) or the proliferation indexes (10.3±0.9 in controls vs. 12.0±1.5 in the Sandostatin-group, N.S.).

Discussion

Our results demonstrate that Sandostatin (octreotide) can induce apoptosis in human pancreatic carcinoma xenografts, not just following a 1-month administration as published earlier (10, 11) but also after a short-term treatment and at a lower dose. These findings indicate that the apoptosis-induced of the somatostatin-analog is an early event and does not require accumulation of the drug.

The spontaneous apoptotic activity in pancreatic cancers is known to be low: Virkäjarvi et al. claimed 0.59-0.69% (14, 15), while others mentioned an apoptotic index of 4.9±4.8 (16). Some tumors are intrinsically resistant and some others are sensitive to apoptosis induction. An increase of apoptosis in these carcinomas can be achieved by many different routes, including eicosapentaenoic acid (17), retinoids (18), virus-mediated wild-type p53 gene (19), and hormonal treatments can also be effective. It was reported that 2-methoxyestradiol inhibited the growth of pancreatic cancer cell lines in a dose-dependent fashion, induced apoptosis and the formation of lung colonies was depressed after i.v. injections in nude mice (20). This hormone also induces the release of cytochrome c into the cytosol and translocation of Bax into the mitochondria (21). Other hormones, e.g., cholecystokinin or gastrin, can increase the level of anti-apoptotic proteins such as bcl-2, but neutralization of the gastrin gene may provide an adjunct to conventional chemotherapy (22).

As for somatostatin analogs in pancreatic cancer, our report was the first to demonstrate apoptosis induction in a hamster model of pancreatic carcinogenesis (23) and these experimental results were subsequently corroborated by others. Using a tumor-selective analog (TT-232), over a 90% growth inhibition was achieved in different pancreatic cancer
cell lines inducing apoptotic cell death (24). In our earlier xenograft models, the apoptosis-induction of octreotide treatment was demonstrated by morphometry, TUNEL-based immunohistochemistry and flow cytometry (10, 11) and it was found that chronic administration on different xenografts doubled the number of tumor cells displaying apoptotic bodies. Similar results obtained when the proportions of the sub-G1 fractions were measured. In those experiments, the evaluation was performed at the end of a 30-day-treatment, but the early effects were not checked. In the present work, the results indicate the same figure: more than a 3-fold elevation in the tumor cells with apoptotic morphology and a doubled proportion of the sub-G1 fraction. Although no data were obtained regarding the successive alterations during chronic octreotide treatment, it is presumable that the increased apoptotic activity remains a constant event during long-term administration of the drug since the early and late results are commensurable.

In pancreatic cancer, the therapeutic resistance is partly due to changes of apoptotic proteins by multiple mechanisms and many efforts have been made to restore apoptosis by various strategic approaches (25). Studies were also performed to examine the significance of this process on survival in pancreatic cancer. The results, although still conflicting, point to the importance of apoptosis in this on survival in pancreatic cancer. The results, although still conflicting, point to the importance of apoptosis in this process (26), but other reports indicated that the contemporary immunohistochemical overexpression of bcl-2 and bax was found to indicate a favorable prognosis (27), as reinforced by TUNEL method results (28).

Today gemcitabine (Gemzar) is the first-line chemotherapeutic drug in pancreatic cancer treatment. Its primary action is the disruption of cellular replication, but the antiproliferative effect is partly due to induction of apoptosis (29). There are reports that gemcitabine treatment also leads to overexpression of somatostatin receptors in different pancreatic cancer cell lines within 4 days following administration (30) and this finding raises the possibility that by using an appropriate schedule, octreotide and Gemzar could enhance each other’s apoptosis-inducing effect.

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References


