Treatment of N-Nitrosobis(2-oxopropyl)amine-induced Pancreatic Cancer in Syrian Golden Hamsters with d-Trp-6-LH-RH and Somatostatin Analogue RC-160 Microcapsules

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ABSTRACT

Antitumoral effects of the agonist of luteinizing hormone-releasing hormone (d-Trp-6-LH-RH) and the somatostatin analog RC-160 (d-Phe-Cys-Tyr-d-Trp-Lys-Val-Cys-Trp-NH₂) on chemically induced ductal pancreatic adenosarcomas were studied. The tumors were induced in female Syrian golden hamsters by weekly s.c. injections of N-nitrosobis(2-oxopropyl)amine at a dose of 10 mg/kg b.w. for 6 weeks. 18 weeks after the last injection, the peptides in controlled-release microcapsule formulations were administered s.c. The animals received the following therapies: Group 1 (N = 15), vehicle only; Group 2 (N = 13), d-Trp-6-LH-RH microcapsules releasing 25 μg/day injected s.c. once a month; Group 3 (N = 14), RC-160 microcapsules, liberating 25 μg/day administered s.c. every 15 days; Group 4 (N = 14), the combination of d-Trp-6-LH-RH plus RC-160 microcapsules. The experiment was terminated on the 80th day when all hamsters in the control group were dead, but in the treated groups 2, 3, and 4, we observed 71, 77, and 86% of survival rate, respectively. In addition to the prolongation of survival, the combination treatment resulted in a significant decrease in the tumorous pancreatic weight, increase in the body weight of the animals, reduction in ascites from 100 to 8.3% and regressive histological changes in 67% of the specimens. Our findings suggest that somatostatin analogues and d-Trp-6-LH-RH could be considered for the development of hormonal therapy for pancreatic cancer.

INTRODUCTION

Exocrine pancreatic cancer has not been considered as a classical hormone-dependent neoplasm, but recent experimental and clinical findings indicate that it may be sensitive to sex steroids, gastrointestinal hormones, and growth factors (1-26). Various experimental results also indicate that the growth of pancreatic tumors can be influenced by hormonal manipulations.

The proliferation of human pancreatic carcinoma Colo-357 cell line cultured in vitro can be stimulated by androgens and inhibited with tamoxifen, progesterone, or Danazol (antiprogesterin and antiandrogen compound) (7). Greenway et al. (8) reported that the growth of human pancreatic adenocarcinoma xenografts in nude mice could be inhibited by the antiandrogen cyproterone acetate and increased by testosterone. Similarly, castration induced a significant decrease in the weight and volume of transplanted ductal pancreatic tumors in Syrian hamsters (9). Several clinical studies indicate that the treatment with antiestrogen tamoxifen resulted in prolongation of survival in patients with pancreatic carcinoma (1, 10, 11).

Gastrointestinal hormones (gastrin, secretin, and cholecystokinin) also seem to be involved in the stimulation of the growth of the pancreatic cancer (18, 22-24). Although early studies did not demonstrate the responsiveness of the chemically induced hamster tumor to secretin (27), later it was found that secretin administered together with BOP3 increased the induction rate and decreased the latency period of pancreatic adenocarcinomas (12). Similar results were obtained by using cholecystokinin (14). Combined injection of secretin and the cholecystokinin-analogue cerulein increased the weight and DNA content of pancreatic ductal adenocarcinoma transplanted into the cheek pouches of male Syrian hamsters (14).

Recently the importance of various endogenous growth factors in the control of neoplastic processes has been demonstrated (15-17, 25, 26, 28). Somatostatin was reported to antagonize the trophic effects of cerulein on pancreas (29). As somatostatin and especially its synthetic analogues can effectively abolish the release and/or action of gastrointestinal hormones (23) and interfere with the action of growth factors (16, 17, 24), these multiple inhibitory effects could be the rationale for its application in the treatment of pancreatic cancer.

Therapy with LH-RH analogues is being utilized to induce the regression of sex-steroid dependent tumors especially prostate cancer (18, 30). In our previous studies we reported the use of LH-RH agonists and somatostatin analogs for the treatment of experimental pancreatic cancer in rats and hamsters (9, 20, 21). In the present study we investigated whether the combination of the agonist d-Trp-6-LH-RH with a powerful somatostatin-analogue RC-160 could enhance the inhibition of BOP-induced pancreatic cancer in hamsters.

MATERIALS AND METHODS

Animals. 70 female Syrian golden hamsters (CR:RGH) weighing 100 ± 10 g were obtained from NCI Frederick Cancer Research Facility (Frederick, MD). They were housed three per cage at the Animal Research Facility in an air conditioned room at 72 ± 2°F and 55 ± 5% humidity, kept under an automatic light/darkness schedule (12/12 h), and given Rodent Laboratory Chow 5001 and tap water ad libitum.

Induction of Pancreatic Cancer. The induction of pancreatic cancer was carried out as described by Pour et al. (31) with some recent modifications suggested by Dr. P. Pour (21). N-Nitrosobis(2-oxopropyl)amine (BOP; ASI-279) was purchased from Ash Stevens, Inc. (Detroit, MI) and stored at 4°C. The required amount was freshly dissolved in 0.9% of NaCl solution and given s.c. into the interscapular region at a dose of 10 mg/kg body weight under a light methoxyflurane (Metofane; Pitman-Moore, Inc., Washington Crossing, NJ) anesthesia. BOP was injected once a week for 6 weeks. 18 weeks later (24 weeks from the start of the experiment) the hamsters were randomly divided into groups and the treatment was initiated.

Peptides. The LH-RH analogue d-Trp-6-LH-RH (pGlu-His-Trp-Ser-Tyr-d-Trp-Leu-Arg-Pro-Gly-NH₂) was synthesized by solid-phase methods and supplied by Debiopharm (Lausanne, Switzerland). Microcapsule formulation of this agonist in biodegradable poly (DL-lactide-co-glycolide) was prepared by Dr. P. Orsolini at Cytotech S.A., Martigny, Switzerland using a phase-separation process. This delayed release...

1 The abbreviation used is: BOP, N-nitrosobis(2-oxopropyl)amine.
calculated by using χ²-test.

capsules were injected on Days 0, 15, 30, and 45. Statistical significance was

RC-160 microcapsules

D-Trp-6-LH-RH microcapsules

N = 15); Group 2, D-Trp-6-LH-RH microcapsules (36 mg/animal, 

designed to release about 25 µg/day of RC-160 for 2 weeks from an 
aliquot of 6.5-mg microcapsules.

Experimental Protocol. Both types of microcapsules were suspended in 0.7 ml of vehicle solution, containing 2% CM-cellulose and 1% Tween 20 in water. This suspension was mixed thoroughly using a Vortex mixer and injected through an 18-gauge needle, s.c. The animals received the following treatments: Group 1, vehicle only (BOP controls; N = 15); Group 2, D-Trp-6-LH-RH microcapsules (36 mg/animal, every 30 days; N = 13); Group 3, RC-160 microcapsules (6.5 mg/animal, every 15 days; N = 14); Group 4, combination treatment that is 6.5 mg/animal of RC-160 microcapsules on Days 0, 15, 30, 45, plus 36 mg/animal of D-Trp-6-LH-RH microcapsules on Days 0 and 30; N = 14). 10 hamsters served as untreated controls. The experiment was terminated on the 80th day of the treatment, when all animals in the BOP control group were dead.

Pathological Procedure. During the treatment period the dead animals showing no autolytic changes were processed for histology. In three animals which died in the 2-week period before the introduction of peptide-treatment, multifocal pancreatic carcinomas were seen. The diameter of the pancreatic nodules varied between 3 to 8 mm. At the end of the experiment a complete autopsy was made. Under metofane anesthesia the ascites was removed, blood was taken from the aorta and caval vein, and the organs were excised, cleaned, and weighed. Half of each pancreatic tumor was frozen at —70°C for receptor assays. The results obtained from receptor assays will be published elsewhere. The remaining tissue was fixed in 8% buffered formalin. The specimens were embedded in paraplast and step sections of 6 µm in thickness were cut. The slides were stained with H&E, periodic acid-Schiff, mucicarmine, toluidine blue, alcianblue-periodic acid-Schiff (pH 2.5) and Mallory-trichrome.

Statistical analyses were made using Student’s t test or χ² test.

RESULTS

Survival. All three therapies resulted in a significant prolongation of survival. At 60 days from the beginning of treatment, 79 to 92% of the treated animals were alive, in contrast to 33% survival rate seen in the untreated group (Table 1). 20 days later all animals in the untreated BOP-control group were dead, but the survival rates in the RC-160, D-Trp-6-LH-RH, and combination groups were 71, 77, and 86%, respectively. These values proved to be highly significant (P < 0.0001) as compared with the BOP controls. However, no statistically significant differences were found between the different treatment groups.

Gross Examination. In the animals with pancreatic cancer and receiving only the vehicle (BOP controls), a significant decrease in body weight was recorded, despite the presence of ascites in all hamsters (Table 2). No significant changes were found in the average body weights in the groups treated with single peptides. However, body weights increased significantly in the group which received RC-160 plus D-Trp-6-LH-RH microcapsules in combination. This synergistic effect is especially noteworthy because only one of these animals had ascites. Consequently, the weight increase was not due to fluid accumulation.

At the carcinomatous stage, the average weight of the tumorous pancreata increased more than 5-fold which was highly significant compared with the pancreata in normal hamsters (P < 0.0005). All three treatment modalities resulted in reduction in pancreatic tumoral weights (Table 2).

Macroscopically, the tumorous pancreata were characteristically multinodular in appearance, with no preferred lobe. The diameters of the individual nodules varied from 2 to 8 mm, but sometimes they were confluent, making it impossible to recognize the original shape of the organ. Because of the multinodularity and in absence of the obvious "normal" pancreatic tissue between the nodules, the weight of tumorous pancreas was recorded. Intraabdominal dissemination occurred in all animals which received no treatment, causing hemorrhagic ascites. All three treatment modalities resulted in a decreased frequency of the peritoneal metastases and the hemorrhagic ascites, but no significant differences in these aspects were seen between the D-Trp-6-LH-RH- and RC-160-treated groups. However, the combination of the two peptides proved to be the most effective and in this group only one out of 12 animals showed bloody abdominal fluid (Table 2).

The weights of various organs are shown in Table 3. The livers in all animals with pancreatic cancer were moderately enlarged (P < 0.025 vs. untreated controls), their surface was finely granular, and characteristically, they contained multiple small fluid-filled cysts. The adrenals in hamsters with pancreatic cancer were larger than those in the normal animals, and this enlargement also occurred in the D-Trp-6-LH-RH- and RC-160-treated groups. However, the combination of both peptides resulted in a smaller increase of the adrenal weights (P < 0.025 vs. BOP controls). The ovaries in the BOP control group were

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Pancreatic weights (g)</th>
<th>Number and (%) of animals with ascites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls (N = 10)</td>
<td>175.6 ± 5.2</td>
<td>0.42 ± 0.02</td>
<td>0/10</td>
</tr>
<tr>
<td>BOP controls (N = 15)</td>
<td>137.0 ± 4.9</td>
<td>2.40 ± 0.27</td>
<td>15/15 (100)</td>
</tr>
<tr>
<td>D-Trp-6-LH-RH microcapsules (N = 10)</td>
<td>142.1 ± 7.6</td>
<td>1.09 ± 0.24</td>
<td>7/10 (70)</td>
</tr>
<tr>
<td>RC-160 microcapsules (N = 10)</td>
<td>131.3 ± 7.3</td>
<td>1.04 ± 0.24</td>
<td>4/10 (40)</td>
</tr>
<tr>
<td>RC-160 plus D-Trp-6-LH-RH microcapsules (N = 12)</td>
<td>164.6 ± 9.1</td>
<td>1.28 ± 0.28</td>
<td>1/12 (8.3)</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM; 4P < 0.005 vs. untreated controls (Student's t test); 5P < 0.005 vs. untreated controls (Student's t test); 6P < 0.001 vs. BOP-controls (Student's t test); 7P < 0.025 vs. BOP controls (χ² test); 8P < 0.01 vs. BOP controls (Student's t test); 9P < 0.025 vs. BOP controls (Student's t test); 6P < 0.001 vs. BOP controls (Student's t test); 10P < 0.005 vs. D-Trp-6-LH-RH-treated group (χ²); 11P = not significant vs. RC-160-treated group (χ²-test).

Table 1 Survival rate of Syrian golden hamsters with BOP-induced pancreatic cancer, treated with vehicle, microcapsules of d-Trp-6-LH-RH and RC-160 or the combination d-Trp-6-LH-RH microcapsules were given on Days 0 and 30. RC-160 microcapsules were injected on Days 0, 15, 30, and 45. Statistical significance was calculated by using χ²-test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival rate at Day 60</th>
<th>Survival rate at Day 80</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP controls (vehicle only)</td>
<td>5/15 (33)</td>
<td>0/15 (0)</td>
</tr>
<tr>
<td>d-Trp-6-LH-RH microcapsules</td>
<td>12/13 (92)</td>
<td>10/13 (77)</td>
</tr>
<tr>
<td>RC-160 microcapsules</td>
<td>11/14 (79)</td>
<td>10/14 (71)</td>
</tr>
<tr>
<td>RC-160 plus D-Trp-6-LH-RH microcapsules</td>
<td>12/14 (86)</td>
<td>12/14 (86)</td>
</tr>
</tbody>
</table>

* Number of the living animals compared to the total number of hamsters in each group.

* P < 0.005 vs. BOP-control.

* P < 0.0001 vs. BOP-control.

* P < 0.025 vs. BOP-control.
significantly smaller than those in the untreated animals \( (P < 0.05) \), but it is not known if this was due to the BOP itself or the induced pancreatic cancer. The D-Trp-6-LH-RH treatment resulted in a further, highly significant decrease in ovarian weights \( (P < 0.0005) \) vs. BOP controls, while in the RC-160-treated animals a noteworthy increase was found \( (P < 0.005) \) vs. BOP group. The combined administration of the peptides led to a similar, but less striking increase.

Microscopic Findings. Because of the autolytic changes in some dead animals, only 44 samples could be evaluated histologically. Pancreatic carcinoma was found in 41 samples (93\%), these neoplasms being invariably of ductal/ductular origin and no acinar cell tumors occurred. All but one proved to be well differentiated forms with various histological patterns (Table 4).

It is noteworthy that all samples, even the largest tumors from the untreated animals were devoid of necrosis, and no regression signs were found. However, in the treated groups the histological regression was clearly determined in 45 to 67\% of the carcinomas, indicating a tumor-inhibiting effect of the peptides (Table 5, Figs. 1–3). The earliest regressive changes induced by the treatment with the analogues were confined to the cytoplasm; the tumor cells became swollen, vacuolized, and ballooning degeneration was seen. These cells occurred in clusters or desquamated into the lumen. Shrinkage, fragmentation, or lysis of the nuclei were also present, but the most frequent change was the karyopyknosis, resulting in small, dark nuclei in the devitalized cells. The tumor cells became flattened, atrophic, with a highly basophilic cytoplasm, and the outlines of the glands were irregular and distorted. In the most advanced stage of the regression the epithelial lining had almost completely disappeared. The lumina contained a variable amount of desquamated cells or debris. In some cases, especially in the group treated with the combination, polymorphonuclear leukocytes and macrophages accumulated around the impaired glandular tumors. Calcified areas appeared in a multifocal pattern. It should be emphasized that these histological regression signs were found only in the treated groups, the combination of D-Trp-6-LH-RH plus RC-160 microcapsules being the most effective. In this group 67\% of the pancreatic carcinomas showed regressive changes, compared with the 45\% in the D-Trp-6-LH-RH group and 56\% in the animals treated with RC-160.

Histological evaluation revealed that metastases were present in 67\% of the animals. Their commonest localization was the liver (36\%) followed by the lungs (22\%). Lymph node and kidney metastases were rarely seen. In this context, it must be mentioned that regression was observed not only in the primary pancreatic tumors but also in some metastatic lesions, being found in three liver and in one kidney metastasis. In these cases, the histological changes were similar or identical to the alterations described above (epithelial atrophy, shrunk, pyknotic nuclei, cellular loss, etc.).

DISCUSSION

The inhibitory effect of D-Trp-6-LH-RH and the early analogues of somatostatin containing 14 amino acids (18, 21, 23, 24) on the growth of the pancreatic cancers in animal models was first demonstrated by Redding and Schally (9). Both types of analogues reduced the growth of acinar pancreatic carcinomas transplanted into rats and ductal cancers grafted into hamsters. Later studies in hamsters with BOP-induced pancreatic cancers confirmed and reinforced these findings (20, 21); the modern somatostatin analogue RC-160 containing eight amino acids (21, 23, 24, 32) and D-Trp-6-LH-RH injected in microcapsule formulations resulted in a prolongation of survival and decrease in tumor weight and volume. In our previous study a histological regression was seen in 28 to 35\% of the treated animals (21). Since these peptides were effective when administered alone, we surmized that the combination treatment could be more efficacious than the therapy with single agents. The present study supports this concept in several aspects. In the group treated with D-Trp-6-LH-RH and RC-160 microcapsules in combination, the weight of the animals was increased, and fewer peritoneal metastases occurred when compared with the groups which received single treatment only. In addition, the survival rate was prolonged and the tumor's pancreatic weight was decreased, although these differences were not significant versus groups treated with single peptides. Histologically, regressive changes were seen in 67\% of the

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Table 3 Organ weights in hamsters with BOP-induced pancreatic cancer treated with D-Trp-6-LH-RH and RC-160 microcapsules

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Liver (g)</th>
<th>Kidneys (g)</th>
<th>Adrenals (mg)</th>
<th>Ovaries (mg)</th>
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<tbody>
<tr>
<td>Untreated controls ( (N = 3) )</td>
<td>7.2 ± 0.4*</td>
<td>1.76 ± 0.02</td>
<td>22.0 ± 3.3</td>
<td>42.0 ± 1.7</td>
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<tr>
<td>BOP controls ( (N = 15) )</td>
<td>10.2 ± 0.9*</td>
<td>1.66 ± 0.1</td>
<td>38.7 ± 2.9</td>
<td>31.0 ± 2.5*</td>
</tr>
<tr>
<td>D-Trp-6-LH-RH microcapsules ( (N = 10) )</td>
<td>9.7 ± 1.2</td>
<td>1.53 ± 0.05</td>
<td>36.0 ± 2.7</td>
<td>23.0 ± 0.9*</td>
</tr>
<tr>
<td>RC-160 microcapsules ( (N = 10) )</td>
<td>10.6 ± 0.8</td>
<td>1.57 ± 0.07</td>
<td>40.4 ± 5.3</td>
<td>44.5 ± 3.1f</td>
</tr>
<tr>
<td>RC-160 plus D-Trp-6-LH-RH microcap-</td>
<td>14.2 ± 2.9</td>
<td>1.69 ± 0.08</td>
<td>31.8 ± 1.8</td>
<td>37.3 ± 1.4f</td>
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</tbody>
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* \( P < 0.05 \) vs. untreated controls; \( P < 0.01 \) vs. untreated controls; \( P < 0.025 \) vs. BOP controls; \( P < 0.005 \) vs. BOP controls. The levels of significance were calculated by using Student's t test.

Table 4 Histological forms of the BOP-induced pancreatic cancer in Syrian hamsters

| Histology                      | BOP
<table>
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</thead>
<tbody>
<tr>
<td></td>
<td>controls</td>
<td>D-Trp-6-LH-RH</td>
<td>RC-160</td>
<td>Combination</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papillary</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mixed ductal/insular</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cystic-papillary</td>
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<td>1</td>
<td>1</td>
<td>2</td>
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</tr>
<tr>
<td>Micronucleous</td>
<td>10</td>
<td>14</td>
<td>10</td>
<td>12</td>
<td>46</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 5 Histological regression changes in the hamster pancreata after treatment with D-Trp-6-LH-RH, RC-160, and combination of both peptides in microcapsules

<table>
<thead>
<tr>
<th>Treatments (%)</th>
<th>No. of histological samples</th>
<th>No. of samples with pancreatic carcinoma</th>
<th>No. of samples with histological regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>BOP controls</td>
<td>9</td>
<td>9</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D-Trp-6-LH-RH microcapsules</td>
<td>11</td>
<td>11</td>
<td>5 (45)*</td>
</tr>
<tr>
<td>RC-160 microcapsules</td>
<td>11</td>
<td>9</td>
<td>5 (56)*</td>
</tr>
<tr>
<td>D-Trp-6-LH-RH plus RC-160 microcapsules</td>
<td>13</td>
<td>12</td>
<td>8 (67)*</td>
</tr>
</tbody>
</table>

* \( P < 0.025 \) vs. BOP controls; \( P < 0.01 \) vs. BOP control; \( P < 0.005 \) vs. BOP controls; \( \chi^2 \)-test. No significant differences were found between the treated groups with respect to histological regression.
HORMONAL TREATMENTS OF HAMSTER PANCREATIC CANCER

Fig. 1. Regressive changes in the pancreatic carcinomas after treatment with D-Trp-6-LH-RH microcapsules. A, tumor cells are swollen, some of them show cytoplasmic vacuolization and shrinkage of the nuclei (× 250). B, clusters of degenerated cells are desquamated into the lumen. The glandular lining is flattened. Around the tumorous gland a large amount of connective tissue and reactive inflammatory cells are seen (× 325). C, cellular debris in the lumina (× 250). D, at the most advanced stage the glandular lining has almost completely disappeared (× 250).

Fig. 2. Regressive histological alterations in the pancreatic tumors after RC-160 microcapsule treatment. Distorted tumoral glands with cellular debris in the lumina. Few leukocytes also appear (× 325).

specimens, more frequently than in the groups which received D-Trp-6-LH-RH or RC-160 alone.

In histopathological terminology, the regressive alterations refer to the morphological changes in the cells and tissues that are characterized by the diminished functions of the cells due to interference with the intracellular metabolic processes. These include both cytoplasmic and nuclear lesions: hydropic, vacuolar, fatty or ballooning degeneration, cytoplasmic homogenization, cellular atrophy, and calcification. The forms of the nuclear destruction are karyopyknosis, karyorrhexis, and karyolysis. At the most advanced stage of the regression, the cells become necrotic. All these regressive changes could be observed in the treated animals, but no such alterations occurred in the untreated group.

The agonistic analogue D-Trp-6-LH-RH, in which the glycine residue in position 6 of LH-RH is replaced by D-Trp, is 100-times more potent than the parent hormone (18, 30). Chronic administration of this analogue produces inhibition of the hypophysis and gonads and causes a reduction in circulating levels of gonadotropins and gonadal steroids (18, 30). The creation of a state of sex-steroid deprivation is the main basis for oncological use of LH-RH in the therapy of androgen or estrogen-dependent tumors. Since pancreatic cancer might be at least in part sex-steroid dependent (1-11, 22), the therapeutic effect of LH-RH-agonists could be explained by elimination of the stimulatory action of the sex steroids. However, other possibilities such as a direct effect on the tumor cells cannot be ruled out. Although Wahlström and Seppälä (33) failed to demonstrate LH-RH-like immunoreactivity in human ductal and acinar pancreas adenocarcinomas, our recent observations indicate the presence of D-Trp-6-LH-RH receptors in BOP-induced hamster pancreatic carcinomas, but not in the normal pancreata.

Modern somatostatin analogues such as RC-160, in addition to their inhibitory effect on the growth hormone release, also block the secretion or action of the gastrointestinal hormones gastrin, secretin, and cholecystokinin (18, 23, 29, 34) and interfere with the stimulatory effects of epidermal growth factor in pancreatic cancer lines (16, 17). A number of somatostatin analogues have been tested in various animal tumor models, including prostate and breast cancer, pancreatic carcinomas, and osteogenic neoplasms, and shown to exert inhibitory effects (9, 20, 23, 24, 32). The exact mechanism of the action of somatostatin analogues also remains to be fully elucidated. For somatostatin analogues there is no clear correlation between the inhibiting activities on release of GH and gastrin and their antitumoral effect. It is possible that some analogues might exert some direct inhibitory effects on tumor tissue (23, 24, 25). In our previous studies on BOP-induced pancreatic carcinomas, histological evidence of regression was obtained in 28% of tumors in hamsters treated with 10 μg/day of RC-160 (21). In the present work we could observe 56% of regression in the
HORMONAL TREATMENTS OF HAMSTER PANCREATIC CANCER

Fig. 3. Regressive changes in the pancreatic tumors after combination treatment with D-Trp-6-LH-RH and RC-160 microcapsules. A, structural disorganization and excessive vacuolar-ballooning degeneration is shown (× 325). B, tumorous glands are lined by thin, devitalized epithelial cells, with shrunken nuclei (× 325). C, macrophage and leukocyte invasion is seen around the vacuolated, individualized, and necrotic tumor cells (× 325). D, structure of the tumor is completely disorganized: necrotic changes are accompanied by reactive mesenchymal cell proliferation, leukocyte-macrophage infiltration, the necrotic tumor cells are floating in the lumina (× 325). E, calcified area in the tumor (× 325).

specimens. This indicates that the increase of the dosage of RC-160 from 10 to 25 µg/day augmented the antitumoral effect of this compound. In another experiment, with an identical design to that described here, the reduction in the dose of RC-160 from 25 µg/day to 15 µg/day decreased the efficacy of treatment-inhibitory response in hamsters with ductal pancreatic carcinoma. Our overall results suggest that to inhibit the growth of pancreatic cancers, relatively large doses of RC-160 might be required.

The microcapsule formulation of the peptides provides a practical and convenient method of avoiding the fluctuating blood levels of the analogues which occur after repeated daily or twice-a-day injections. The administration of the peptides formulated in biodegradable poly(DL-lactide-co-glycolide) microcapsules assures a controlled, continuous release and therapeutic blood levels. It has also been demonstrated, that the tumor-inhibitory effect of D-Trp-6-LH-RH microcapsule formulations is superior to that of the unencapsulated form (35). The microcapsules of RC-160 have been perfected very recently (36).

Combined administration of D-Trp-6-LH-RH and the RC-160 proved to be more effective in inhibiting the growth of experimental prostate and breast cancer than when these peptides were given alone (24, 37-39). However, this combination has not been investigated previously in pancreatic cancer models and this study provides the first account of its efficacy. Our work reinforces the concept that pancreatic carcinoma is sensitive to hormonal manipulations. Preliminary clinical results indicate that patients with pancreatic cancer respond to D-Trp-6-LH-RH (19), antiestrogen tamoxifen (10, 11), or to RC-160 (24), but the combination therapies have not been used so far. Our studies suggest that the combination D-Trp-6-LH-RH and RC-160 in microcapsules for delayed release might possibly be of value in the treatment of pancreatic cancer.

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Treatment of $N$-Nitrosobis(2-oxopropyl)amine-induced Pancreatic Cancer in Syrian Golden Hamsters with d-Trp-6-LH-RH and Somatostatin Analogue RC-160 Microcapsules

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