Effects of new bombesin antagonists given singly or in combination with a somatostatin analog on nitrosamine-induced pancreatic cancers in hamsters

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Abstract. In three experiments, hamsters with N-nitroso-bis(2-oxopropyl)amine-induced pancreatic cancers were treated for two months with bombesin/GRP antagonists RC-3095 [D-Tpi^6,Leu$^{13}\psi$(CH$_2$NH)Leu^{14}-bombesin(6-14)], RC-3910-II [D-Tpi^6,Leu$^{13}\psi$(CH$_2$N)Tac^{14}-bombesin(6-14)], RC-3940-II [Hca^6,Leu$^{13}\psi$(CH$_2$N)Tac^{14}-bombesin(6-14)], RC-3950-II [D-Phe^6,Leu$^{13}\psi$(CH$_2$N)Tac^{14}-bombesin(6-14)], somatostatin analog RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH$_2$), or the combination of RC-3095 with RC-160. All peptides inhibited pancreatic cancers to various degrees, reducing the number of tumorous animals, lowering the weight of tumorous pancreata by 40-55% and decreasing AgNOR numbers which are indicators of cell proliferation rate. Combination therapy with RC-3095 and RC-160 did not inhibit tumors better than single peptides. Among new bombesin/GRP antagonists, RC-3940-II had the strongest inhibitory effect. RC-3950-II and RC-3095 caused similar inhibition, but RC-3910-II was less effective. Tumor inhibitory activity of the bombesin/GRP antagonists was correlated with their binding affinities to bombesin receptors on tumor cells. RC-3940-II caused 50% inhibition of specific binding of [^{125}I-Tyr^4]bombesin to tumor cell membranes at 0.96 nM concentration, while the IC$_{50}$ for RC-3950-II was 5.27 nM and 12.94 nM for RC-3095. Our findings suggest that in addition to RC-3095, other bombesin/GRP antagonists such as RC-3950-II and especially RC-3940-II could be further developed for therapy of human pancreatic cancer.

Introduction

Bombesin and related peptides like gastrin releasing peptide (GRP) exert diverse physiological and pharmacological effects on pancreas (1-3). On the basis of well established hormonal activities, the role of bombesin-like peptides in pancreatic carcinogenesis has emerged and is now supported by much experimental data. Receptors for GRP were detected in CAPAN human pancreatic cancer cells (Avis FP, *et al*, Proc Am Assoc Cancer Res 29: abs.54, 1988). Bombesin promoted azaserine-induced pancreatic cancers in rats (4) and stimulated growth of CFPAC-1 human pancreatic cancers *in vitro* (5). Caerulein, cholecystokinin (CCK) and secretin, which can mediate some effects of GRP, enhanced the carcinogenic effects of nitrosamines on pancreas of hamsters (6-8). mRNAs for both bombesin and its receptor protein were detected in various human pancreatic cancer cell lines, which supports the concept that GRP might be an autocrine growth factor in pancreatic cancers (9).

In view of a possible involvement of bombesin/GRP in pancreatic cancer, we developed a series of bombesin/GRP receptor antagonists, and evaluated them in various models of experimental pancreatic tumors (10-12). Pseudononapeptide bombesin/GRP antagonist RC-3095 significantly inhibited growth of nitrosamine-induced pancreatic cancers in hamsters (13) and inhibited *in vitro* and *in vivo* growth of CFPAC-1 human pancreatic cancer cells (5). The inhibitory effect of the bombesin/GRP antagonist could not be nullified by administration of bombesin or GRP (14). We also detected that the inhibition of pancreatic cancers by bombesin analogs was accompanied by a decrease in binding capacity of EGF-receptors in tumor membranes (13-15). Thus the effects of these bombesin/GRP antagonists could be mediated by interference with EGF-receptor mechanisms.

In order to improve therapeutic results, we developed new, more active bombesin/GRP antagonists (16,17). Another approach to improve antitumor action of bombesin/GRP antagonists would be to use them in combination with other hormone analogs for treatment of pancreatic tumors.

A combination therapy with bombesin/GRP antagonist and LH-RH analogs was tested previously (15). LH-RH agonists and antagonists alone showed inhibitory effect on experimental pancreatic cancers. The therapeutic action of LH-RH analogs could be explained in part by the creation of a state of the sex steroid deprivation. However, a direct effect of LH-RH analogs on pancreatic cancers is also possible (11,12). Surprisingly, the tumor inhibitory effect of the combination therapy with bombesin/GRP antagonist and

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LH-RH analogs was not superior to that of single agents, although these peptides have different receptors and utilize apparently different mechanisms of action (15).

In this study, we analyzed the effects of another possible combination therapy on nitrosamine-induced pancreatic cancers in hamsters. This is a standard model of human pancreatic carcinoma of ductal origin. Bombesin antagonist RC-3095 was administered together with somatostatin analog RC-160. The latter peptide was also found to inhibit experimental pancreatic tumors (18,19). Because of some differences in their mechanisms of action, additive antitumor effect could be expected. We also compared the effects of several newer bombesin/GRP antagonists with those of RC-3095, which was used in many earlier experiments.

Materials and methods

Peptides. Bombesin receptor antagonist D-Tpi^{6},Leu^{13} ψ(CH_2NH)Leu^{14}-bombesin(6-14) (RC-3095), originally synthesized in our laboratory (20) was made by Asta Pharma (Frankfurt/M, Germany). Tpi (2,3,4,9,-tetrahydro-1H-pyrido(3,4-b) indol-3-carboxylic acid) is a conformationally constrained analog of Trp and is more hydrophobic than Trp. RC-3095 trifluoroacetate (D21663) was used for treatment of hamsters. New bombesin antagonists, RC-3910-II [D-Tpi^{6},Leu^{13}ψ(CH_2N)Tac^{14}-bombesin(6-14)], RC-3940-II [Hca^{6},Leu^{13}ψ(CH_2N)Tac^{14}-bombesin(6-14)], and RC-3950-II [D-Phe^{6},Leu^{13}ψ(CH_2N)Tac^{14}-bombesin(6-14)] were synthesized in our laboratory by solid-phase methods (16). Hca is desaminophenylalanine and Tac is thiazolidine-4-carboxylic acid. Somatostatin analog RC-160 (D-Phe-Cys-Tyr-D-Trp-Lys-Val-Cys-Trp-NH_2) originally synthesized by solid-phase methods and evaluated in our laboratory, was made by classical synthesis by Novabiochem, Laufelfingen, Switzerland. RC-160 acetate Lot no. A 07609 was used for the group treated with combination. Microgranules of RC-160 pamoate in poly(DL-lactide-co-glycolide) were prepared by Cytotech S.A., Martigny, Switzerland. Batch no. RCSER-91-15M was designed to release about 35 μg/day of RC-160 for 10 days from an aliquot of 0.6 mg microgranules.

In experiments I and II, peptides were administered by osmotic pumps (Alza Corp. Palo Alto, CA). Model 2ML4, releasing 2.5 μl/h for 4 weeks was implanted s.c. The filling of pumps and the implantation were described, and the injection method of microcapsules was also reported earlier (13). In experiment III, peptides were dissolved in 0.1% dimethyl sulfoxide (DMSO) and in saline. The animals were injected s.c. with 0.5 ml peptide solution once daily.

Animals and tumors. Two hundred and twenty female Syrian golden hamsters (CH:RGH) weighing about 100 g were obtained from National Cancer Institute, Frederick Cancer Research Facility (Frederick, MD). The animals were maintained and pancreatic tumors were induced with N-nitroso-bis(2-oxopropyl)amine (BOP) (American Tokyo Kasei, Portland, OR) as previously described (13).

Experimental protocol. Three experiments were performed, and the BOP injected hamsters received the following treatments:

Experiment I: Group 1, Injection vehicle only (BOP-controls); group 2, RC-3095 20 μg/day; group 3, RC-160 35 μg/day; group 4, RC-3095 20 μg/day + RC-160 35 μg/day.

Experiment II: Group 1, Injection vehicle only (BOP-controls); group 2, RC-3095 5 μg/day; group 3, RC-3950-II 5 μg/day; group 4, RC-3910-II 20 μg/day; group 5, RC-3940-II 20 μg/day.

Experiment III: Group 1, Injection vehicle only (BOP-controls); group 2, RC-3095 30 μg/day; group 3, RC-3940-II 30 μg/day; group 4, RC-3950-II 30 μg/day; group 5, Untreated healthy hamsters.

The groups consisted of 7-13 hamsters as shown in Table I. The time-schedule of the experiments, the autopsy and histological procedures were the same as described (13,14).

Serum growth hormone (GH), insulin-like growth factor-I (IGF-I), epidermal growth factor (EGF), and gastrin levels. Serum GH was determined by RIA using materials provided by the National Hormone and Pituitary Program (NHPP, Rockville, MD) (rat GH-RP-2/AFP-3910B; rat GH-I-6/AFP-5676B; and rat GH RIA-5 antibody/AFP-411S) (14,21). IGF-I and EGF were extracted by the modified acid-ethanol method (22). The extracted IGF-I was measured by RIA using IGF-I (a gift from Genentech) for standard in the range of 2-500 pg/tube. Antibody UB3-189 (a gift from Dr L. Underwood and Judson J. Van Wyk) distributed by NIDDK was used in the final dilution of 1:10,000. The extracted EGF was measured using mEGF standard (receptor grade, UBI, Lake Placid, NY) in the range of 0.006-12.2 ng/tube and mEGF antiserum provided by Collaborative Research (Bedford, MA) in the final dilution of 1:167,000. Serum gastrin levels were measured using RIA Kit provided by Becton-Dickinson (Orangeburg, NY).

Receptor studies. Tumor cell membranes were prepared and [Tyr^{4}]bombesin was labeled with ^{125}I as described previously (23). Inhibition of [^{125}I-Tyr^{4}]bombesin binding to membranes of nitrosamine-induced pancreatic cancers by various concentrations of the bombesin antagonists RC-3095, RC-3940-II and RC-3950-II was tested in 2-3 independent experiments, each performed in triplicate. IC_{50} was calculated by a computerized curve fitting program (24) from the displacement experiments. IC_{50} is defined as the dose causing 50% inhibition of specific binding of [^{125}I-Tyr^{4}]bombesin to the tumor membranes.

Statistical analyses. Statistical evaluation of data was performed by Duncan's multiple range test and Student's t-test by using a computer program.

Results

Body and organ weights, survival of hamsters. In experiment I, body weights of animals receiving RC-160 were 30% less than those of the control group. Organ weights were not changed by the treatments. Body and organ weights did not differ from control values in experiment II. In experiment III, body weights of the hamsters did not differ significantly from control values. Weights of livers, hearts, kidneys, spleens and sex organs (ovaries and uteri) were similar among groups.

Table I. Effect of treatment with bombesin/GRP antagonists on BOP-induced pancreatic cancers in hamsters.

Treatment	Number of tumorous animals/total hamsters	Dead hamsters with those dying of tumors in brackets	Invasive tumor	Weight of pancreas (g)	Weight of tumorous pancreata (g)	% decrease in tumorous pancreas weight
Experiment I						
1. Control	12/12	6 (6)	4	2.53±0.80	2.53±0.80	0
2. RC-3095 20 µg/day	6/12	7 (2)	3	0.77±0.18[2]	1.33±0.26	47
3. RC-160 35 µg/day	6/11	2 (1)	2	0.79±0.22[2]	1.12±0.35	56
4. RC-3095 + RC-160	8/12	4 (1)	3	0.72±0.14[2]	0.84±0.20[1]	67
Experiment II						
1. Control	10/11	5 (4)	5	1.26±0.29	1.35±0.31	0
2. RC-3095 5 µg/day	7/11	4 (1)	1	0.56±0.14	0.73±0.19	46
3. RC-3950-II 5 µg/d	5/11	3 (1)	1	0.55±0.19	0.80±0.36	41
4. RC-3910-II 20 µg/day	10/11	2 (2)	2	0.75±0.14	0.76±0.15	44
5. RC-3940-II 20 µg/day	7/11	2 (0)	0	0.60±0.17	0.67±0.11[1]	50
Experiment III						
1. Control	11/13	4 (4)	4	1.83±0.66	2.07±0.76	0
2. RC-3095 30 µg/day	12/13	3 (3)	1	1.13±0.29	1.19±0.31[1]	43
3. RC-3940-II 30 µg/day	12/13	1 (1)	1	0.63±0.14[2]	0.64±0.15[2]	69
4. RC-3950-II 30 µg/day	12/13	3 (3)	3	1.04±0.21[1]	1.10±0.22[2]	47
5. Untreated healthy	0/0	2 (0)	-	0.49±0.02[2]		

Values are means ± S.E. [1]$p<0.05$; [2]$p<0.01$.

However, average liver weight of all animals receiving BOP was higher than that of healthy hamsters. Ovarian and uterine weights of BOP-treated hamsters were lower than those of untreated animals ($p<0.05$), except for the group treated with RC-3940-II, in which ovarian weights reached the values of healthy hamsters.

The number of animals that died during the treatment showed great variations among groups (Table I). However, fewer hamsters with pancreatic cancer died in all treated groups.

Tumor pathology. In spite of the standardized conditions provided for all experimental animals, there were some variations in the frequency and sizes of pancreatic tumors among different experiments due to unknown causes. Thus treated groups should be compared always with the control group of the same experiment. The average weight of the pancreata was reduced in all treated groups by 69-71% in experiment I, by 40-55% in experiment II, and by 38-66% in experiment III. Because of the multinodularity of cancers and the confluence of nodules, the weight of tumorous pancreata (including both tumor weight and the weight of the rest of the pancreas) was recorded on the basis of histological examination. The average weight of the control tumorous pancreata was 2.53 g, 1.36 g and 2.07 g in experiments I, II, and III respectively (Table I). Weights of tumorous pancreata were reduced in the treated groups by 47-67%, 41-50%, and 43-69% in experiments I, II and III respectively (Table I).

Histologically, the pancreatic tumors were adenocarcinomas with various types and degree of differentiation, as described earlier (25). There were no significant differences in histological pattern or the occurrence of cystic lesions and the amount of stroma between treated and control groups. The quantitative histological data are shown in Table II. The frequency of mitosis and apoptosis in tumors was expressed by the percentage of tumorous glandular structures containing mitotic cell or apoptotic alterations, as described earlier (13). The argyrophilic nucleolar organizer region (AgNOR) numbers are good indicators of cell proliferation rate in tumors (26). The number of mitotic cells was decreased in all treated groups, but this change was significant only in group 2 of experiment I. The frequency of apoptosis was similar in treated and control tumors. However, the AgNOR numbers were decreased in all tumors treated with bombesin/GRP antagonists.

Serum GH, IGF-I, EGF and gastrin levels. GH and IGF-I levels in serum were decreased after therapy with RC-160 alone, but not when the somatostatin analog was given in combination with RC-3095. None of the bombesin/GRP antagonists caused significant changes in serum EGF, IGF-I, GH or gastrin levels. Data are shown in Table III.

Receptor assays. All three bombesin/GRP antagonists tested (RC-3095, RC-3940-II and RC-3950-II) inhibited the binding of [^{125}I-Tyr^4]bombesin to membranes of nitrosamine-induced pancreatic cancers. IC_{50}, i.e. the concentration causing 50%

Table II. Effect of treatment with bombesin/GRP antagonists on histological cell proliferation characteristics of BOP-induced pancreatic cancers in hamsters.

Experiment group	% of gland exhibiting mitosis	% of glands exhibiting apoptosis	Number of AgNORs per cell
Experiment I			
1. Control	10.0±1.9	29.7±3.0	8.84±0.33
2. RC-3095 20 µg/day	3.7±0.3[1]	37.0±6.1	7.83±0.12[1]
3. RC-160 35 µg/day	8.2±3.9	36.3±7.2	7.88±0.37
4. RC-3095 + RC-160	4.6±1.4	27.9±3.2	8.01±0.33
Experiment II			
1. Control	9.1±1.7	17.4±2.5	7.88±0.24
2. RC-3095 5 µg/day	9.0±2.8	28.0±3.8	6.94±0.24[1]
3. RC-3950-II 5 µg/day	4.8±1.5	15.3±3.3	6.65±0.19[2]
4. RC-3910-II 20 µg/day	5.2±1.0	14.4±2.0	6.24±0.25[2]
5. RC-3940-II 20 µg/day	8.4±2.7	13.1±2.2	6.30±0.19[2]
Experiment III			
1. Control	9.3±3.0	14.3±3.4	6.48±0.28
2. RC-3095 30 µg/day	5.4±1.1	12.2±2.1	5.55±0.15[1]
3. RC-3940-II 30 µg/day	7.3±2.0	14.9±2.3	5.31±0.31[2]
4. RC-3950-II 30 µg/day	8.0±1.9	13.8±2.2	5.64±0.18[1]

Values are means ± SE. [1]$p<0.05$; [2]$p<0.01$.

Table III. Effect of treatment with bombesin/GRP antagonists on serum GH, IGF-I, EGF and gastrin levels of hamsters bearing BOP-induced pancreatic cancers.

Experiment group	Serum GH ng/ml	Serum IGF-I ng/ml	Serum EGF ng/ml	Serum gastrin pg/ml
Experiment I				
1. Control	2.44±0.46	659±144		79.7±13.8
2. RC-3095 20 µg/day	2.49±0.51	395±102		59.3±10.0
3. RC-160 35 µg/day	1.80±0.41[1]	290±82[1]		124.8±25.9
4. RC-3095 + RC-160	2.53±0.27	554±128		120.1±22.6
Experiment III				
1. Control	6.14±0.68	543±44	7.23±0.74	
2. RC-3095 30 µg/day	8.40±0.92	444±52	8.50±0.84	
3. RC-3940-II 30 µg/day	5.95±0.66	447±53	5.09±0.68[1]	
4. RC-3950-II 30 µg/day	6.42±0.58	528±37	5.99±0.66	
5. Untreated healthy hamsters	14.16±1.33[2]	587±21	7.28±1.21	

Values are means ± SE. [1]$p<0.05$; [2]$p<0.01$.

inhibition of specific binding of [^{125}I-Tyr^4]bombesin, was 12.94±0.26 nM for RC-3095; 5.27±0.44 nM for RC-3950-II and 0.96±0.13 nM for RC-3940-II.

Discussion

Pancreatic cancer is the ninth most common malignancy in the United States and represents the fourth most common cause of cancer-related death (27). Despite a continuous search for new diagnostic methods, about 90% of all patients with pancreatic cancer have tumors that are unresectable at the time of diagnosis (27). Conventional chemotherapy alone or in combination with radiotherapy has very little effect on advanced tumors, and the survival of patients with pancreatic cancers has practically not improved in the last decades.

Recent discoveries of genetic alterations in cancer cells and the detection of specific growth factors and hormones that are important in the development of different tumors, among them pancreatic cancers, can lead to new therapeutic approaches (11,28,29). Somatostatin analogs suppress the secretion and/or action of gastrointestinal hormones (gastrin, secretin and cholecystokinin) and decrease GH and IGF-I levels in blood (11,12,28). Somatostatin analogs inhibited growth of experimental pancreatic tumors (13,21,30,31) and decreased the binding capacity of EGF receptors on pancreatic cancer cells of hamsters (13). It was suggested that when RC-160 binds to its receptor, it may stimulate tyrosine phosphatase in the membrane, which in turn dephosphorylates and inactivates EGF receptors (32). Somatostatin analogs were also tried clinically in patients with advanced inoperable pancreatic cancers and in some led to improvement in quality of life and temporary stabilization of the disease (12,33). The present view is, however, that somatostatin analogs alone may not be sufficient for therapy for pancreatic cancer and should be used in combination with other drugs or hormone analogs (12,33).

In view of recent observations that bombesin or related peptides might play a role in pancreatic carcinogenesis, various bombesin/GRP antagonists were synthesized in our laboratory (16,20). Pseudononapeptide antagonist RC-3095 inhibited growth of BOP-induced pancreatic cancers in hamsters (13-15). The growth of human pancreatic cancers SW-1990 and CFPAC-1 in nude mice or their proliferation *in vitro* were also inhibited by RC-3095 (5,34). The tumor inhibitory action of bombesin antagonists was invariably linked to a down regulation of EGF receptors in tumors (13-15). This effect might be mediated through specific receptors for bombesin/GRP detected on tumor cells (5,13,16).

Previously, we obtained encouraging results using combinations of different hormone analogs for treatment of experimental pancreatic tumors. Combination of LH-RH agonist with somatostatin analog resulted in a stronger tumor inhibition than the peptides alone (21,31). Similarly, somatostatin analog RC-160 given together with LH-RH antagonist SB-75 (Cetrorelix) or 5-FU was more effective than single agents in inhibition of BOP-induced pancreatic cancers (35). However, combinations of different peptide hormone analogs do not always result in additive tumor inhibitory effects. Administration of LH-RH analogs together with bombesin/GRP antagonist RC-3095 suppressed BOP-induced pancreatic cancers in hamsters to a similar extent as the single agents (15).

In the present study, in experiment I, a combination treatment with bombesin/GRP antagonist and somatostatin analog was tried. Both analogs alone caused a decrease in the number of tumorous animals, pancreatic weights, the number of mitotic cells and AgNOR counts in tumors. However, tumor growth inhibition produced by the combination was not better than that obtained with single peptides by most parameters and only the decrease in tumorous pancreas weights was greater in the group treated with the combination. The lack of synergism in the effect of somatostatin analogs and bombesin/GRP antagonists is difficult to explain, as these peptides are assumed to have different mechanisms of action. Somatostatin and its analogs could counteract the gastrointestinal hormones that might promote growth of pancreatic cancer and also decrease GH level in blood which in turn would inhibit the production of growth factors such as IGF-I that appear to be involved in proliferation of pancreatic cancer cells (12). The action of somatostatin analogs on cancers can be also explained in part by direct effects on tumor cells mediated by specific somatostatin receptors detected on cancer cells (19,36).

Bombesin-like peptides bind to specific receptors that were detected in BOP-induced pancreatic cancers (13) and in CAPAN human pancreatic cancer cell line (Avis FP, *et al*, Proc Am Assoc Cancer Res 29: abs.54, 1988). The inhibitory effect of bombesin antagonists on pancreatic cancers was linked to a major down regulation of EGF receptors on tumor cells (13,14). The mechanism of the transregulation of EGF receptors by bombesin/GRP antagonists is not clear. It was shown that bombesin enhanced phosphorylation of EGF receptors (37) and antagonist RC-3095 inhibited this effect, but several other mechanisms might be also involved in the transmodulation of EGF receptors by bombesin antagonists, and need to be elucidated.

The binding sites for both somatostatin and bombesin/GRP are G-protein associated receptors. These peptides share common mechanisms in intracellular signaling pathways. Both types of peptides decreased the binding capacity of EGF receptors in tumors. It is possible that secondary messenger systems are maximally utilized by the treatment with single agents and intracellular Ca^{++} stores and ATP sources become exhausted and therefore the effects cannot be increased by combined therapy. One of the indirect effect of somatostatin analogs on tumors can be manifested by decreased blood GH and IGF-I levels. Treatment with RC-160 resulted in a decrease in GH and IGF-I levels, but these changes were not found in the animals treated with the combination.

In summary, the lack of additive effect of a somatostatin analog and a bombesin/GRP antagonist might be possibly explained by interferences with intracellular mechanisms as well as interactions in extratumoral pathways. However, the exact mechanisms of action and interference have to be elucidated.

The inhibitory effect of bombesin/GRP antagonist RC-3095 on tumor growth was demonstrated in several experimental pancreatic tumor models (5,13,14,34). In this study, we also analyzed the effects of other bombesin/GRP antagonists synthesized in our laboratory on the hamster pancreatic cancer model, and compared the results with those of RC-3095. According to the results of previous pilot experiments with RC-3950-II, this compound was tested at a low, 5 μg/day, dose in experiment II, and its tumor inhibitory effect was similar to that of RC-3095. Two other new compounds, RC-3910-II and RC-3940-II, were also compared at a usual 20 μg daily dose. RC-3940-II was superior in almost all parameters tested. In the group receiving RC-3940-II, the number of hamsters that died with tumor during the experiment was the smallest, and there were no invasive tumors seen macroscopically. The pancreatic weights, tumorous pancreas weights and AgNOR numbers were the lowest among groups in experiment II. In experiment III, the three bombesin/GRP antagonists were

compared at somewhat higher, 30 μg/day doses. Again the lowest tumorous deaths, pancreatic weights, tumorous pancreas weights and AgNOR numbers were found in the group that was treated with RC-3940-II. The results of the comparison of the four bombesin/GRP antagonists can be summarized as follows: RC-3910-II was less efficacious than RC-3095 in tumor inhibition. RC-3950-II had about the same effect as RC-3095 and RC-3940-II had the strongest inhibitory action on BOP-induced pancreatic cancers.

In recent experiments, RC-3940-II powerfully inhibited SW-1990 human pancreatic cancer cells proliferating *in vitro* or growing in nude mice. This inhibitory effect was also stronger than that of RC-3095 (34). Thus, relatively small changes in the molecular structure of bombesin antagonists can augment or decrease tumor inhibitory effects. Apparently, these effects can be correlated with binding characteristics of the peptides to specific receptors on cells. Among a large number of bombesin/GRP antagonists developed in our laboratory, RC-3940-II showed 50 times higher binding affinity to receptors on CFPAC-1 cells than RC-3095 (16). RC-3940-II also exhibited higher binding affinities to Swiss 3T3 cells than RC-3095 or RC-3950-II (17). Previously, we detected one class of high affinity binding sites for [^{125}I-Tyr^4] bombesin in membranes of BOP-induced pancreatic cancers (13). In the present study, displacement experiments showed that the antagonists had different binding affinities to the receptors on these cancer cells. The three bombesin antagonists could be arranged in the following order of potencies according to their IC_{50} values: RC-3940-II <RC3950-II<RC-3095. Thus the tumor inhibitory effect of the bombesin antagonists correlated well with the binding affinity of the compound to pancreatic cancer cells.

Bombesin receptors on cancers need to be better characterized. Different tumors may have receptors with diverse binding affinities for various bombesin/GRP antagonists. Our studies show that for treatment of experimental pancreatic cancer RC-3095, RC-3950-II and above all RC-3940-II were the best among the bombesin antagonists presently available. RC-3095 and RC-3940-II might be further developed for therapy of human pancreatic cancer.

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