Pancreatic Carcinogenesis: Effect of Secretin in the Hamster-Nitrosamine Model

Allan G. Howatson and David C. Carter

ABSTRACT—The effect of exogenous secretin on pancreatic carcinogenesis in WO strain hamsters has been examined in the nitrosamine-ductular adenocarcinoma model. Secretin, 20 clinical U/kg, stimulated a maximal secretory response of pancreatic juice and bicarbonate when given iv. The same dose given sc for 6 weeks had no significant effect on pancreatic wet weight and DNA or RNA contents. However, when given to animals receiving N-nitrosobis(2-oxopropyl)amine [(BOP) CAS: 60599-38-4] (5 mg/kg), it reduced the latency and increased the induction rate of tumor development when compared with the carcinogen given alone to animals (secretin + BOP, 15 of 17 animals with tumors; BOP alone, 4 of 13 with tumors at 15 wk; P<.002). These effects are consistent with secretin acting as a cocarcinogen in this model of pancreatic carcinogenesis.—JNCI 1987; 78:101-105.

Numerous epidemiologic studies have demonstrated associations that support the view that carcinoma of the exocrine pancreas in humans is the result of chemical carcinogenesis. Cigarette smoking doubles the risk of developing pancreatic carcinoma (1, 2) and is well recognized as a source of carcinogenic compounds (3, 4). Particular dietary patterns, especially those typical of “Western” societies, have also been implicated as factors increasing the risk of pancreatic tumor development (5-7). The interpretation of these and other associations in the framework of the classical two-stage concept and the current multistep concept of chemical carcinogenesis (8) has led to examination of pancreaticotrophic factors as potential promoters or cocarcinogens in pancreatic carcinogenesis.

Gastrointestinal hormones released from the duodenal mucosa in response to food and gastric acid are mediators of pancreatic exocrine function. CCK acts primarily as a stimulator of the acinar cells, whereas secretin is a stimulant of ductal and ductular cells (9). Both hormones increase the metabolic activity and possibly the turnover rate of their target cells, and the potency of chemical carcinogen is known to be increased in metabolically active and proliferating cell populations (10). We have already demonstrated that pancreatic carcinogenesis in the hamster-nitrosamine model of Pour et al. (11) is enhanced by CCK (12). Given that cancer in this model is of ductular morphology and may have ductular cell origin, the present series of experiments were undertaken to examine the effects of exogenous secretin on carcinogenesis.

MATERIALS AND METHODS

The animals used in all studies were male, WO strain, Syrian golden hamsters kept under standardized conditions in groups of 4 and fed Oxoid 41B diet and water ad libitum.

Effect of secretin on pancreatic exocrine function.—Six hamsters (age, 14 wk; mean body wt, 122±3.3 g) were anesthetized ip with pentobarbital sodium (Sagatal; May and Baker Ltd., Dagenham, England) after a 24-hour fast with free access to water. Tracheostomy was performed and a cannula was inserted iv into the left jugular vein. A saline infusion (0.9%) at a rate of 0.375 ml/hour was commenced with the use of a syringe infusion pump. A laparotomy was performed through a midline incision, the common bile duct ligated in continuity just distal to the entry of the cystic duct, and the pylorus ligated. The common bile duct was cannulated, as it passed through the wall of the duodenum, to permit collection of pancreatic juice. The cannula (2FG; outer diameter, 0.63 mm; Portex Ltd., Hythe, England) was secured by a suture and led out through the flank. The body temperature was maintained at 34°C with a heating pad.

An equilibration period of 1 hour was allowed before commencement of the test. Pancreatic juice was collected in preweighed tubes placed below the hamster. A basal output was collected for 1 hour, and thereafter stepwise increasing doses of secretin (KabiVitrum, Stockholm, Sweden) in saline were infused. Collections at each dose level were made for 30 minutes, and 20 minutes were allowed for equilibration between doses. The volume output was determined by weighing and the bicarbonate content was measured by use of the Natelson microgasometer to determine CO₂ content in 10-μl aliquots of pancreatic juice. At the end of each test, a solution of lissamine green dye was injected up the cannula. A test in this experiment (expt 1) was regarded as

ABBREVIATIONS USED: BOP = N-nitrosobis(2-oxopropyl)amine; CCK = cholecystokinin; CU = clinical units; LSC = liquid scintillation counting; MFO = mixed-function oxidase; PWW = pancreatic wet weight.

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3 University Department of Pathology, Glasgow Royal Infirmary, Castle St., Glasgow, G4 OSF, Scotland. Address reprint requests to Dr. Howatson at this address.
4 University Department of Surgery, Glasgow Royal Infirmary.
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Technically satisfactory if the whole gland was stained, suggesting that all of the pancreas had been draining into a patent duct system and that the entry of the pancreatic ducts into the common bile duct had not been occluded by the cannula.

Trophic effects of secretin.—A) In the second experiment, groups of 10 animals each, 10 weeks of age, at the start of the study received either 20 CU secretin/kg (body wt) twice daily, at 8 a.m. and 6 p.m., for 5 days/week for 6 weeks in hydrolyzed gelatin carrier (mean body wt, 93±6.3 g) (group 1 of exp 2) or gelatin carrier alone (mean body wt, 89.4±4 g) (group 2 of exp 2) by sc injection. The secretin was made up in 10% hydrolyzed gelatin in saline to prolong absorption (13). The dose of secretin used (20 CU/kg) was selected from the above dose-response study as that producing a maximal response in terms of pancreatic juice volume and bicarbonate output when given iv.

At the end of the experiment, the animals were killed; for each animal, the pancreas was removed, trimmed of fat and connective tissue, and weighed. Tissue from two sites in both the gastric and splenic lobes of the pancreas was fixed in Formalin, examined histologically after staining with hematoxylin and eosin, and assessed by means of the same criteria as in the carcinogen experiment. In addition to recording the PWW (PWW, mg pancreas/100 g body wt), the pancreatic contents of DNA and RNA (µg/100 mg PWW) were also determined. The DNA and RNA were extracted from the homogenized pancreas by the method of Schmidt-Thanhauser as modified by Munro and Fleck (14). DNA was measured by the modified Burton method (15,16). RNA was measured by determining the absorbance at 260 nm and 292 nm of the final perchloric acid extract and by employing the formula derived by Fleck and Begg (17).

B) Another experiment (exp 3) to investigate the effect of secretin on pancreatic DNA synthesis, as measured by [3H]thymidine incorporation, was also performed. Three groups of 6 hamsters each (age, 10 wk) received 3 days of treatment with either 100 CU secretin/kg (body wt) at 8 a.m. and 6 p.m. (mean body wt, 99.7±9.64) (group 1 of exp 3) or secretin 20 CU/kg (mean body wt, 98.1±8.2 g) (group 2 of exp 3) or gelatin carrier alone (mean body wt, 101.5±6.9 g) (group 3 of exp 3) by sc injection. One hour after the last of the six injections, each animal received an ip injection of [3H]thymidine (3 µCi/g). The animals were killed 1 hour later; the pancreas was removed, trimmed of fat and connective tissue, and weighed. Approximately 200 mg pancreatic tissue from each animal was prepared for LSC. The pancreas was digested in sodium hydroxide, 1 µl for each milligram of tissue, at 80°C for 1 hour. After cooling, 0.5-ml aliquots were taken and 0.3 ml hydrogen peroxide and 0.3 ml concentrated hydrochloric acid were added. Finally, 15 ml scintillant (SSC3; Koch Light Laboratories, Ltd., Colnbrook, England) was added and the specimen underwent LSC.

Effect of secretin on carcinogenesis.—We entered 175 hamsters 10 weeks of age into this study (exp 4). They were divided in 2 groups. In the first group of experiment 4, the carcinogen BOP (CAS: 60599-38-4) (Ash Stevens, Inc., Detroit, MI) was given to 100 hamsters (mean body wt, 89.9±5.5 g) in a weekly dose of 5 mg/kg for life by sc injection, and 20 CU secretin/kg (body wt) was administered sc in hydrolyzed gelatin carrier for 5 days/week for 6 weeks on the day of carcinogen injection and on the subsequent 2 days. In the second group of experiment 4, 75 hamsters (mean body wt, 90.4±8.9 g) received only the carcinogen BOP in gelatin vehicle in the same dosage as that of group 1 and no secretin. The carcinogen was administered at 8 a.m. (for groups 1 and 2) and the secretin (for only group 1) at 9 a.m. and 6 p.m. on day 1. On days 2 and 3 the secretin injections were given at 8 a.m. and 6 p.m. Secretin was administered over a 6-week period because this was the earliest stage at which well-developed neoplastic microscopic lesions had been identified in pilot studies of the model. All animals were weighed twice/week for the duration of the study.

Cohorts of 20 animals from the secretin plus BOP group and 15 animals from the BOP-alone group were scheduled to be killed at intervals of 5, 7.5, 10, 12.5, and 15 weeks after the start of the experiment. A full postmortem examination was performed, and all major organs were examined macroscopically for the presence of neoplastic lesions. The pancreas was fixed en bloc in 10% Formalin and the whole organ was blocked, then being an average of 11 blocks from each pancreas. Three sections were taken from each block for examination by light microscopy after staining with hematoxylin and eosin. Macroscopic tumor nodules, where present, were excised post mortem from the main pancreatic specimen and separately sectioned for histologic confirmation.

Each pancreas was assessed for the presence of the following histologic appearances: ductal cell dysplasia, ducal carcinoma in situ, atypical ductular proliferation and pseudoductule formation affecting an entire lobule, and pancreatic adenocarcinoma. If any of these ductal-ductular lesions were present in a histologic section, it was recorded as present for that pancreas. No formal attempt was made to quantify the extent or frequency of the lesions in the pancreas. For the purposes of the histologic assessment, ductal cell dysplasia and carcinoma in situ were reported separately. Mitoses had to be present before an atypical hyperplastic lesion was regarded as carcinoma in situ. The proliferation of ductules showing atypical features, usually coexisting with the formation of pseudoductules, was regarded as a sign of a neoplastic lesion with premalignant potential if almost all of a lobe was involved in the process. This display was reported as panlobular ductular proliferation in the histologic assessment. Intralobular carcinoma was defined as a focus of carcinoma confined within a pancreatic lobule. When the lobular boundary was transgressed, the lesion was classified as a frankly invasive carcinoma. For reporting purposes, both forms of carcinoma have been classified under the single heading "adenocarcinoma."

All sections were examined by a single observer (A. G. H.); and approximately 25% of the slides, randomly
selected by an individual not involved in the series of the experiments, were examined by a trained pathologist who was not aware of the nature and duration of treatment given to any particular animal.

Statistical analysis.—All data are expressed as mean ± SD. Dose-response curve data were analyzed by Student's t-test for paired observations. Student's t-test for non-paired values was employed for the analysis of the results of the trophism experiments, the data being normally distributed. The analysis of the histologic assessment was by Fisher's exact probability test.

RESULTS

Effect of Secretin on Pancreatic Exocrine Function

In experiment 1, all six tests were technically satisfactory. The basal output of pancreatic juice was 1,223±206 μL/kg/hour and increased sequentially to a maximum of 2,131±219 μL/kg/hour with infusion of secretin at a dose of 10 CU/kg/hour (P<0.005). The output of bicarbonate in the pancreatic juice rose from 78.4±21.4 μmol/kg/hour to a maximum of 246.7±51 μmol/kg/hour with a dose of 20 CU/kg/hour of secretin (P<0.001). The volume and bicarbonate dose-response curves are shown in text-figures 1 and 2.

Trophic Effects of Secretin

A) In experiment 2, 6 weeks of treatment with secretin did not produce a significant difference in PWW, total pancreatic DNA, or total pancreatic RNA content between the 2 groups (Tested for PWW at mg pancreas/100 g body wt: control group, 295.6±61; secretin group, 266.3±32.2. Tested for DNA content at μg/100 mg PWW: control group, 217.5±29.5; secretin group, 234±37.2. Tested for RNA content at μg/100 mg PWW: control group, 215.6±33; secretin group, 192.8±16.5).

Histologic examination of sections from two sites in both the gastric and splenic lobes of the pancreas of animals receiving secretin for 6 weeks and the corresponding controls showed no abnormalities and, in particular, no evidence of neoplastic lesions.

B) In experiment 3, there was no significant difference in [3H]thymidine uptake among the 3 groups of animals. The results expressed as counts/minute/milligram of pancreatic tissue were: 100 CU secretin/kg, 289.4±43.2; 20 CU secretin/kg, 277±67.5; and control, 257.4±34.8.

Effect of Secretin on Carcinogenesis

A total of 10 animals died before completion of the study (expt 4), 5 in the secretin + BOP group and 5 in the BOP-alone group. At postmortem no animals in either of these treatment groups showed evidence of macroscopic tumors in any organ other than the pancreas and its draining lymph nodes. Metastatic tumor was present in the lymph node draining the gastric and duodenal lobes in 3 animals in the secretin + BOP group at 15 weeks.

Examination of the histologic sections of the pancreas of animals killed at 5 and 7.5 weeks showed no adenocarcinomas and no significant difference between the 2 treatment groups (expt 4) in terms of the development of ductal lesions or panlobular ductular proliferation. At 7.5 weeks, 50% of the animals in each of these 2 groups showed duct dysplasia; duct carcinoma in situ was present in 30% of the secretin + BOP group compared with 20% of the carcinogen-alone group. There was no evidence of panlobular ductular proliferation in either group at 5 weeks, but by 7.5 weeks this was present in 20% of the carcinogen-alone group and in 30% of the secretin + BOP group.

The results of the histologic assessments of the groups at 10, 12.5, and 15 weeks are shown in table 1. At 10 weeks, although there was a numerical excess of ductal lesions and panlobular ductular proliferation in the secretin + BOP group as compared with the findings for the BOP-alone group, this result was not statistically significant. Of the secretin + BOP-treated animals, 3 had developed adenocarcinomas at 10 weeks. At 12.5
ductular lesions, including adenocarcinomas, in 95% of assessors on the presence and development of ductal and animals.

...duct carcinoma in situ, panlobular ductular proliferation... The final measure of the effectiveness of secretin as a cocarcinogen in pancreatic carcinogenesis remains in its effect on latency and tumor induction rate. Secretin significantly potentiated this effect in that ductal cell dys-
plasia and that carcinoma in situ appeared to develop earlier. Panlobular atypical ductular proliferation, which we have regarded as a significant neoplastic lesion with premalignant potential, was present in 45% of the secretin + BOP group and in only 25% of the BOP-alone group at 10 weeks, the difference becoming more pronounced by 12.5 and 15 weeks, respectively. Adenocarcinoma appeared earlier and with greater frequency in the secretin + BOP-treated group than in the group receiving BOP alone. The reduction in the latent period and the increased induction rate of tumor development are consistent with secretin acting as a cocarcinogen in this model.

The mechanism of this action does not appear from the present study to be simply the provision of increased numbers of susceptible cells and intracellular targets for carcinogen following increased cell proliferation. Secretin is a stimulator of ductal and ductular cells as evidenced by its effect on pancreatic juice and bicarbonate output. Clearly, secretin must stimulate the metabolic processes of this cell population to produce an increase in output. This raises the possibility that the action of secretin may be mediated by an effect on the handling of carcinogen by the ductal and ductular cells.

Nitrosamines, including BOP, are indirect carcinogens and have to be metabolized to produce mutagenic-carcinogenic forms. It is now established that nitrosamines are activated by inducible MFO enzyme systems present in the hamster pancreas (24). Induction of these enzymes, known to be present in both ductal-ductular cells and acinar cells (25), is associated with increased conversion of carcinogens to mutagens. The action of secretin might include stimulation of these MFO enzymes, which metabolize BOP as part of a general enhancement of cellular activity. An increase of carcinogen metabolism in situ, with increased production of electrophilic radicals in close proximity to important targets could enhance carcinogenesis either by point mutations (26, 27) or by direct DNA strand breakage (28).

The experiments reported here have shown that secretin, a stimulator of the ductal-ductular cell population, enhances the effect of carcinogen on the hamster pancreas.

REFERENCES