Pancreatic Cancer in the Syrian Hamster Induced by \(N\)-Nitrosobis(2-oxopropyl)amine: Cocarcinogenic Effect of Epidermal Growth Factor

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ABSTRACT

Because epidermal growth factor (EGF) is rapidly bound and internalized into rat pancreas, stimulates uptake of tritiated thymidine, and increases pancreatic weight, a cocarcinogenic effect on pancreatic cancer seemed likely. Pancreatic adenocarcinomas were induced in 70 female Syrian hamsters by 19 weekly s.c. injections of \(N\)-nitrosobis(2-oxopropyl)amine (BOP) (10 mg/kg). From Wk 5 through Wk 8 of BOP injections, additional s.c. injections of EGF (5 \(\mu\)g every 3 days for 10 injections) were given to 45 animals, while 25 received saline solution. An additional group of 10 received EGF alone, and another 10 animals received saline solution alone (controls). Eleven wk later, the mean body weight of EGF-treated animals increased by 29% as compared with that of controls, and their mean pancreatic weight relative to body weight increased by 44% as compared with controls. The mean body weight of EGF + BOP-treated animals increased by 10%, and their pancreatic weight relative to body weight increased by 22% as compared with that of animals treated with BOP alone. The incidence of pancreatic cancer in the EGF + BOP-treated animals was 75% versus 44% in those treated with BOP alone (\(P = 0.016\)). No tumors developed in either animals treated with EGF alone or control animals. EGF augments pancreatic carcinogenesis induced by BOP. The incidence of bronchial carcinomas doubles.

INTRODUCTION

Pancreatic cancer induced in the Syrian hamster by BOP shows many similarities with human pancreatic cancer, including histology and distribution; production of weight loss, diabetes, vascular thrombosis, and peripheral invasion; and the presence of tumor markers (1). EGF is a potent mitogen, which might be expected to potentiate chemical carcinogenesis. Among its effects are shortening of the latency of methylcholanthrene-induced cutaneous cancer in mice (2) and acceleration of growth of a variety of cultured neoplasms derived from epithelium (3, 4). EGF increases rat pancreatic weight and stimulates incorporation of tritiated thymidine (5). Cultured human pancreatic carcinoma cells (PANC-1) incorporate and process EGF (6), and in addition, EGF promotes phosphorylation of another line of human pancreatic cancer cells (PaCa-2) (7). Effects of EGF on chemically induced pancreatic cancer in hamsters were therefore examined.

MATERIALS AND METHODS

Female Syrian hamsters (Charles River Laboratories, Wilmington, MA; \(n = 90\); age, 5 wk) were housed with a 12-h light-dark cycle and were given free access to Purina Laboratory Chow and water. BOP (Ash Stevens, Detroit, MI) was dissolved in normal saline solution before administration, and EGF (culture grade; Collaborative Research, Inc., Lexington, MA) was administered from a fresh aqueous solution of 20 \(\mu\)g/ml.

Animals were divided among 4 groups: EGF + BOP (\(n = 45\)); BOP only (\(n = 25\)); EGF only (\(n = 10\)); and controls, which were given saline solution (\(n = 10\)). After being acclimatized, the groups to receive EGF + BOP or BOP only were each given a course of 19 weekly s.c. injections of BOP (10 mg/kg) in 0.9% saline solution, while the group to receive EGF only received equivalent volumes of 0.5 ml of saline solution. From Day 28 through Day 55 after the start of the BOP course, s.c. injections of 5 \(\mu\)g of EGF in 0.25 ml of saline solution were given every 3 days to the EGF + BOP group and the EGF groups, for a total of 10 injections. Animals assigned to receive BOP only received the same volume of saline solution. Control animals received injections of saline solution alone.

To detect pancreatic cancer early, 3 animals from the EGF + BOP group were sacrificed by an overdose of ether for autopsy examination at wk 8, 12, 16, and 18 of BOP treatment (12 animals). In addition, to those deaths, one animal in the EGF + BOP group died after 12 wk, and one in the control group, after 13 wk. Three more animals treated with EGF + BOP died at 18 wk, but autolysis prevented their examination. The remaining 73 animals were sacrificed after 19 wk of BOP or saline treatment for autopsy examination.

Each pancreas was removed by dissection of its 3 lobes from surrounding structures. After being trimmed of peripancreatic fat, pancreases were weighed and fixed in 10% formalin. The anatomical surfaces of each lung, liver, and kidney, and sections cut at 0.5-cm intervals through each of these organs were examined for abnormalities. Areas suspected of being cancer were removed and fixed in 10% formalin. Pancreatic sections were cut at 5-\(\mu\)m intervals, the number of sections from each pancreas varying according to the size of the organ and to the number of visible tumors. All tissues were stained with hematoxylin and eosin for histological examination on coded slides.

The diagnosis of pancreatic cancer was made according to the criteria of Cubilla and Fitzgerald (8). The presence of pancreatic cancer was recorded for each organ without trying to count the number of tumors in each organ, because sections through a single neoplasm were often multiple. Pancreatic cancers were defined as "local" when they were confined to the pancreas (Fig. 1) or "invasive" when they infiltrated peripancreatic fat, connective tissue, or lymph nodes.

Statistical analyses were generally made using the \(\chi^2\) test. Analysis of variance with mean separation analysis was used for comparison of pancreatic and body weights (9).

RESULTS

Seventy-three animals (81%) survived until the end of the experiment. Results from them and from 3 animals sacrificed at 18 wk were included (76 animals).

Hamsters treated with EGF alone had weights similar to those of control animals, but they were 8% heavier than both groups receiving BOP at the time EGF treatment began (Fig. 2). At the end of the experiment, those receiving EGF alone were 29% heavier than control animals, 36% heavier than animals receiving BOP alone, and 24% heavier than EGF + BOP-treated animals. EGF-treated animals weighed more than controls after 1 wk of EGF injections, and EGF + BOP-treated animals weighed more than those receiving BOP alone after wk 9 of BOP treatment (\(P < 0.01\)).

At the end of the experiment, the mean pancreatic weight relative to body weight of EGF-treated animals was 44% more than that of control animals and 22% more than that of animals
receiving BOP alone (Table 1). The mean pancreatic weight relative to body weight of EGF + BOP-treated animals was also 22% more than that of animals receiving BOP alone. Greater pancreatic cellularity, reflecting an increased number of dysplastic ductal foci, and an increased number of tumors in the pancreas of EGF + BOP-treated animals contributed to the increase in pancreatic weight of this group compared with that of animals receiving BOP alone.

Three of the 12 animals sacrificed before 19 wk of BOP treatment developed pancreatic adenocarcinoma. After 19 wk of BOP injections, 75% of the 32 animals receiving EGF + BOP had local pancreatic cancer, compared with 44% in animals treated with BOP alone ($P = 0.016$) (Table 2). There was no statistical difference in the incidence of pancreatic cancers showing invasiveness between the EGF + BOP group (63%) and the BOP group (73%), however.

The incidence of bronchial adenocarcinomas was greater in the EGF + BOP-treated group (56%) than in the group treated with BOP alone (28%) ($P = 0.03$) (Table 2). In addition, there were 2 extrapancreatic tumors: a large hemangioma of the liver in one EGF + BOP-treated animal and a metastatic pancreatic adenocarcinoma in the liver of another in the same group. No tumors were found in control animals and none in animals receiving EGF without BOP.

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**Table 1** Effect of EGF on pancreatic weight after 19 weekly injections of BOP or saline solution

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals</th>
<th>Pancreatic wt (mg)</th>
<th>Pancreatic/body wt (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>10</td>
<td>851 ± 31*$^{a}$</td>
<td>3.99 ± 0.19</td>
</tr>
<tr>
<td>BOP</td>
<td>25</td>
<td>512 ± 30</td>
<td>3.28 ± 0.20</td>
</tr>
<tr>
<td>EGF + BOP</td>
<td>32</td>
<td>687 ± 29</td>
<td>3.99 ± 0.14$^{b}$</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>466 ± 31</td>
<td>2.78 ± 0.09$^{c}$</td>
</tr>
</tbody>
</table>

*$^{a}$ Mean ± SE.
*$^{b}$ Including 3 animals sacrificed at 18 wk.
*$^{c}$ $P < 0.01$ (comparison with BOP or controls).
*$^{d}$ $P < 0.01$ (comparison with EGF, EGF + BOP, or BOP).

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**Table 2** Incidence of pancreatic and bronchial adenocarcinomas after EGF treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of animals with local or invasive pancreatic cancers$^{*}$</th>
<th>No. % of total</th>
<th>No. of animals with bronchial cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGF</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BOP</td>
<td>25</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td>EGF + BOP</td>
<td>32</td>
<td>24$^{*}$</td>
<td>63 $^{*}$</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>35</td>
<td>66 $^{*}$</td>
</tr>
</tbody>
</table>

*$^{*}$ As defined in the text, local cancers were confined to the pancreas; invasive cancers infiltrated peripancreatic fat, connective tissue, or lymph nodes.

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**Figure 1** Photomicrograph of a poorly differentiated adenocarcinoma of the pancreas arising from a large pancreatic duct (curved arrow) in a hamster treated with EGF + BOP. Although a tumor replaces most of the pancreatic tissue, there are an area of preserved acinar pancreas (arrow at upper left) and an area of preserved islet tissue (arrow at upper right). H & E, × 20.

**Figure 2** Body weight of EGF-treated and saline-treated animals during 19 wk of BOP treatment or saline injections.
DISCUSSION

EGF injected as described in these experiments almost doubles the incidence of pancreatic and bronchial adenocarcinomas induced by BOP in Syrian hamsters of the Charles River strain. A cocarcinogenic effect is apparent, but not whether EGF modifies initiation of carcinogenesis or promotes it. Although pancreatic cancers are produced in Eppler Institute hamsters by a single injection of BOP (1), Charles River hamsters require a minimum of 4 injections (10 mg/kg) for a 25% incidence after 30 wk. In rats a single injection of N-nitrosobis(2-hydroxypropyl)amine can cause pulmonary adenocarcinoma specifically (10), but BOP as used in the present experiments causes both pancreatic and bronchial cancers.

Compared with the pancreatic and body weights of hamsters treated with BOP only, the addition of EGF for 10 doses over about 4 wk, as used in these experiments, increases body weight by 10% and pancreatic weight relative to body weight by 22%. Inasmuch as the absolute dose of BOP varies directly with weight, the heaviest animals (those receiving EGF) received the largest amounts, and this variable could have contributed to the increased incidence of cancer. In human cancers, actuarial records show an increased incidence as body weight increases (11). Conversely, extreme weight loss in rats tends to decrease susceptibility to chemical carcinogenesis (12, 13).

Although weight gain may favor carcinogenesis, the mitogenic action of EGF on somatic growth and pancreatic acinar cell growth is more likely to be responsible for the effects observed in the hamster, inasmuch as cellular proliferation is essential for carcinogenesis and potentiates it (14, 15), and EGF is trophic for a variety of untransformed cultured cells, including fibroblasts (16, 17), glial cells (18), mammary epithelial cells (19), chondrocytes (20), kidney cells (21), and hepatocytes (22); for cultured human pancreatic ductal cells (6, 7), colonic adenocarcinoma cells (3), and breast cancer cells (4); and in vivo for mouse mammary glands (23) and rat gastrointestinal mucosa and pancreatic acinar cells (5). While binding of EGF to its receptors on cell membranes may mediate these mitogenic effects directly, interaction or synergy of EGF with a second anabolic hormone such as insulin could stimulate organ and body growth (24, 25).

EGF increases the incidence of anal cancers induced by 1,2-dimethylhydrazine in mice more than 300% (26). EGF and other trophic factors from male mouse salivary glands may favor colonic carcinogenesis, inasmuch as removal of the mouse submandibular glands, a rich source of EGF and other growth factors, decreases the incidence of dimethylhydrazine-induced colonic cancer from 71% to 47% (27).

Both EGF and PDGF are polypeptide hormones that bind to specific cell surface receptors and promote cellular proliferation (28–31). The inclusion of the oncogenes V-erb-B or V-sis in the genomes of EGF or PDGF target cells may lead to expression of cell surface receptors that are similar to the receptors for EGF or PDGF (32–34). Recognition of EGF or PDGF by these altered oncogene-encoded receptors could lead to unregulated growth of target cells. Although there is no evidence for the inclusion of oncogenes in pancreatic cancer cells in the Syrian hamster, BOP could activate a latent oncogene, causing expression of receptors similar to those for EGF. Extrinsically administered EGF could then stimulate unrelated cell growth after binding to them.

ACKNOWLEDGMENTS

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REFERENCES


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