Induction of Exocrine Pancreatic, Bile Duct, and Thyroid Gland Tumors in Offspring of Syrian Hamsters Treated with N-Nitrosobis(2-oxopropyl)amine during Pregnancy

Parviz M. Pour

Eppley Institute for Research in Cancer, University of Nebraska Medical Center, Omaha, Nebraska 68105

ABSTRACT

We examined the effect on the Syrian hamster fetal pancreas of N-nitrosobis(2-oxopropyl)amine (BOP), a potent pancreatic carcinogen in adult hamsters. Pregnant Syrian hamsters (F₀ generation) were treated with BOP (10 mg/kg body weight) at the 8th, 10th, 12th, and 14th days of gestation (for a total dose of 40 mg/kg body weight). Treatment was well tolerated and all hamsters delivered, at term, pups (F₁ generation = 24 females and 27 males) with no abnormalities in number per mother or in physical and behavioral conditions, when compared to matched F₁ controls (20 females and 17 males). The experiment was terminated when hamsters in each group (F₀ and F₁) were 46 weeks old. Pancreatic tumors were found in 89% of the BOP-treated F₀ generation and in 5 (50%) of their male litters, but none was seen in their female progeny or in any hamsters from the F₁ control group. Tumors in the BOP-treated F₀ generation hamsters were ductular adenomas (78%), ductular carcinomas in situ (11%), and ductal/ductular carcinomas (3%). Tumors in their litters were ductular adenomas (20%), ductular carcinomas in situ (10%), and poorly differentiated tumors (20%) that resembled human pancreatic toplastomas. The incidence of common duct polyps (44%), gallbladder polyps (44%), and cholangiomas (44%) was significantly higher in the BOP-treated F₀ generation than in their litters (which had incidences of 10, 0, and 40%, respectively). Pulmonary and renal neoplasms occurred only in the F₁ generation, whereas ovarian and thyroid gland neoplasms were found only in the F₁ generation. Results indicate a differing susceptibility of fetal and maternal tissues to BOP.

INTRODUCTION

Syrian hamsters present a unique model for pancreatic carcinogenesis (1). Among several pancreatic carcinogens tested, BOP² was shown to be the most potent for the hamster pancreas (1,2) and to have a selective affinity for the pancreas as compared with other related compounds (2). It is not known if the affinity of BOP for the pancreas depends on the stage of tissue maturation. Transplacentally, pancreatic neoplasms have not been induced by any of the carcinogens related to BOP (3-7), but the effects of BOP on the developing pancreas have not been investigated. Since the pancreases of young animals have been shown to be more responsive to carcinogenesis than are those of adults (8), it was of interest to examine the response of the fetal hamster pancreas to BOP, by treating pregnant hamsters during different gestation periods (from the 8th through 14th day of gestation) with BOP and comparing its carcinogenic effects in parents and their offspring.

MATERIALS AND METHODS

Carcinogen. BOP was synthesized and purified as described (9).

Animals. Syrian golden hamsters from the Eppley colony were used. Twenty females, all 8-10 weeks old with an average body weight of 110 ± 15 (SD) g, were mated with healthy males of a similar age. Pregnancy was confirmed through vaginal smears, and the first day of mating was regarded as the first day of pregnancy (gestation). The pregnant hamsters were divided into 2 groups. One group (group F₀-1) was treated with BOP (10 mg/kg body weight s.c.) at the 8th, 10th, 12th, and 14th days of gestation. Group 2 consisted of saline-treated controls (F₀-2). After delivery, the number of pups per mother, the physical and behavioral conditions, and weight of the pups were recorded. Litters (F₁ generation) were removed from their mothers at 4 weeks of age and placed in plastic cages in groups of 5 by sex. All animals were maintained under standard laboratory conditions and given pelleted diet (Allied Mills, Chicago, IL) and tap water ad libitum. They were checked twice daily and weighed every 2 weeks. All hamsters were sacrificed at 46 weeks of age.

They were necropsied completely, and their external and internal organs were carefully examined for abnormalities. All tissues were fixed in 10% buffered formalin, and the following were processed for histology by conventional methods and stained with hematoxylin and eosin: nasal and paranasal cavities; larynx; trachea; pharynx; esophagus; stomach; small and large intestines; gallbladder; common duct; pancreas; liver; kidneys; urinary bladder; urethra; ovaries; uterus; vagina; prostate; Cowper's glands; testes; thyroid; parathyroid; and adrenal glands. Pancreas were cut into step sections (4 sections/organ), and pancreatic tumors were also stained by immunoperoxidase-antiperoxidase techniques to assess the presence of insulin, glucagon, and somatostatin and to demonstrate A blood group antigen, as described (10). The durations stated correspond to the animals' ages. Hamsters that died before the age of 20 weeks were excluded from the study. Induced pancreatic lesions were diagnosed as described (1,10). For statistical evaluation, the data in the mother hamsters were compared with those of the litters in the group instead with those of individual F₁ generation, because each offspring was not individually treated. The χ² test was used for statistical evaluation.

RESULTS

The number of F₀ and F₁ generation hamsters in each group, their average body weights and survival, and the patterns and sites of tumors are listed in Table 1. All litters from BOP-treated dams (F₀-1) were born at term and showed no physical or behavioral abnormalities when compared to the controls (F₀-2); also there were no differences in the number of pups/mother and survival between these 2 groups. However, the offspring of both sexes in the BOP-treated group were 20-30% heavier than those of untreated controls, and the reasons for this difference could not be determined. All but 7 females in group F₁-1 and 7 females and 8 males in group F₁-2 survived for 46 weeks.

Pancreatic Tumors. Of the BOP-treated dams (F₀-1) 8 (89%) had pancreatic neoplasms. The tumors in 7 of the animals were ductular adenomas. One animal also had a ductular carcinoma in situ, and there were 3 adenocarcinomas, measuring 5-10 mm in diameter. In the F₁-1 group (BOP-exposed litters), 5 (50%) male litters, but no females, had pancreatic tumors; of these, 2 hamsters had ductular adenomas, 1 ductular carcinoma in situ, and 2 poorly differentiated tumors (8 and 10 mm in diameter), both of which were located in the body of the pancreas splenic lobe. Both of the latter tumors had similar histological patterns.
TUMOR INDUCTION BY BOP

Table 1 Effect of BOP on F₀ and F₁ generation of hamsters

<table>
<thead>
<tr>
<th></th>
<th>BOP group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F₀-2</td>
<td>F₁-2</td>
</tr>
<tr>
<td>No. of litters</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>No. of pups</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. of dams</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Av. survival</td>
<td>38 ± 6^</td>
<td>44 ± 4</td>
</tr>
<tr>
<td>Av. body wt</td>
<td>104 ± 25</td>
<td>167 ± 33</td>
</tr>
<tr>
<td>Pancreas lesions</td>
<td>8 (89%)^p</td>
<td>0</td>
</tr>
<tr>
<td>Ductular adenoma</td>
<td>7 (78%)^p</td>
<td>0</td>
</tr>
<tr>
<td>Ductular carcinoma in situ</td>
<td>1 (11%)^p</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>3 (33%)</td>
<td>0</td>
</tr>
<tr>
<td>“Pancreatoblastoma”</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholangioma</td>
<td>4 (44%)^d</td>
<td>4 (40%)</td>
</tr>
<tr>
<td>Common duct polyp</td>
<td>4 (44%)^d</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Gallbladder polyp</td>
<td>4 (44%)^d</td>
<td>0</td>
</tr>
<tr>
<td>Lung adenoma</td>
<td>2 (22%)</td>
<td>0</td>
</tr>
<tr>
<td>Renal adenoma/carcinoma</td>
<td>2 (22%)</td>
<td>0</td>
</tr>
<tr>
<td>Vagina papilloma</td>
<td>1 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid gland adenoma</td>
<td>0</td>
<td>5 (50%)</td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>39 ± 8</td>
<td>44 ± 7</td>
</tr>
<tr>
<td>P &lt; 0.005 compared with F₀-1 female.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.05 compared with F₁-1 male.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.05 compared with F₀-1 female.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>* Ovarian granulosa cell tumor.</td>
<td></td>
<td></td>
</tr>
<tr>
<td># Foregut polyp.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Undifferentiated portion of pancreatic tumor "pancretoblastoma" in male offspring of BOP-treated hamster dam. Partial lobular formation of tumor cells by connective tissue bands. Focal inflammatory reaction (top and lower right). Mitotic figures (for examples, see arrowheads) can readily be identified by this magnification. H & E, × 78.

Fig. 2. The tumor in Fig. 1 (top) has invaded the "capsule" (large arrowheads) and adjacent acinar cells (large arrow). Mitotic figures (small arrows). H & E, × 200.

and were composed of pleomorphic cells arranged in lobular or haphazard patterns (Fig. 1). Focal invasion of the surrounding exocrine tissue was evident (Fig. 2). The tumor cells were oval or round, imitating ductular or immature insular cells. Several distended, minute, or abortive glandular structures could be discerned (Fig. 3). Scattered between the tumor cells were a few insulin-, glucagon-, and somatostatin-containing cells. No cells of an acinar nature were found. The luminal portion of the epithelium and mucin of the glandular or cystic structures

3664
reacted positively with anti-A sera, as did the cytoplasm of a few tumor cells in poorly differentiated areas (Fig. 4).

All pancreatic tumors in the F₁-1 group were found in animals that were sacrificed at 46 weeks of age; pseudoductular formations were found in 14 of these hamsters (7 females and 7 males), but only in 3 control hamsters (F₁-2; 2 females, 1 male). Focal atypical proliferation of islet cells occurred in 1 F₁-1 hamster and hepatocyte-like cell metaplasia in 3 F₁-1 hamsters, but none in other groups. These metaplastic cells developed primarily in the periphery of islets. Other, more common pancreatic lesions found were intrainsular ductules in F₀-1 and F₁-1 hamsters; however, none were observed in other groups.

DISCUSSION

The present study demonstrates that the fetal pancreas is apparently able to metabolize BOP directly or react with BOP metabolites generated elsewhere in the fetal or maternal tissue. The finding of a specificity of BOP (or its metabolites) for the fetal pancreas is enhanced by the failure of BHP to similarly induce pancreatic tumors transplacentally, even with a much higher dose of the carcinogen (100 mg/kg body weight) and a much longer survival period for the hamsters (82 ± 24 weeks) (5), as compared to 46 weeks in this study.
factor also dictates the carcinogenic response of the fetal tissues.

The histomorphological and immunohistochemical similarities between pancreatic lesions in the F₀ and F₁ generations indicate a similar histogenesis for pancreatic tumors in the adult and fetal pancreas.

Two of the pancreatic tumors in the F₁ group were of a poorly differentiated type and resembled, in some respects, human pancreaticoblastomas of a mixed type (17), which are not seen in adult hamsters (1, 10, 18). However, contrary to the findings in humans (17, 19, 20), no acinar cells were found in these tumors. On the other hand, in analogy to human tumors, endocrine cells were an integral part of these neoplasms. In this context when one considers the occurrence of human pancreatic tumors in some family members (21) and in children of parents with certain occupations (22), the transplacental effect of some carcinogens could be one of the etiological factors in human pancreatic cancer, especially in view of the assumption that the development of human pancreaticoblastomas is the result of oncogenesis during early embryonic life (17).

Development of thyroid follicular adenomas in the present study is of great interest, since such tumors have not yet been induced in adult hamsters by BOP, but have occurred in a high incidence in adult rats (23) and were occasionally found in the hamster offspring treated with related nitrosamines (5). Consequently the fetal thyroid gland in hamsters is apparently as responsive to the carcinogenicity of BOP as are those of adult rats. However, this responsiveness is somehow lost in mature hamster tissue.

REFERENCES

Induction of Exocrine Pancreatic, Bile Duct, and Thyroid Gland Tumors in Offspring of Syrian Hamsters Treated with $N$-Nitrosobis(2-oxopropyl)amine during Pregnancy

Parviz M. Pour


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/7/3663

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.